

# **EXHIBIT 1**



## In Sequence 2013 Survey: Illumina Pulls Further Ahead, Interest in Oxford Nanopore Remains High

Jan 07, 2014 | Julia Karow

**An end-of-year** sequencing survey of GenomeWeb readers reveals that Illumina has solidified its dominant market position in next-gen sequencing over the last year.

The overall picture holds true for the clinical sequencing market as well, assessed for the first time this year, where Illumina's leadership is even more pronounced.

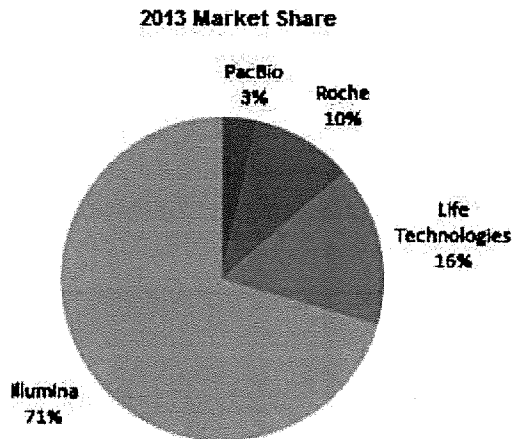
When it comes to purchasing plans, Illumina is rivaled by Oxford Nanopore Technologies, which recently started an early access program for its first commercial platform.

In addition, the majority of survey responders expects the next big technology advance in sequencing to come from Oxford Nanopore, followed by Illumina and Pacific Biosciences.

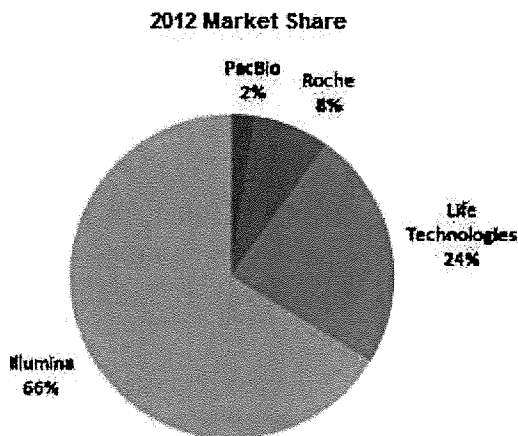
The survey, *In Sequence's* 6th annual assessment of the next-generation sequencing market, was conducted in late December in collaboration with investment firm Mizuho Securities, which analyzed the data. Questions were sent out via e-mail to a subset of GenomeWeb's readership. One hundred and forty-nine readers completed the survey in full or in part, of which 121 said they currently use next-gen sequencing.

About two-thirds of respondents have at least one MiSeq, and more than half own at least one HiSeq, illustrating Illumina's market leadership. About two-fifths have at least one PGM, and 12 percent at least one Ion Proton, both of which are sold by Life Technologies' Ion Torrent. Approximately one-fifth each own a Roche 454 system or at least one PacBio RS, about 10 percent have at least one Illumina Genome Analyzer, and fewer than 10 percent own one or more Life Tech SOLiDs.

Based on an estimate of the number of sequencing systems owned by survey participants, Illumina holds about 70 percent of the overall market, gaining slightly at the expense of its competitors compared to our 2012 survey. Life Technologies lost 8 percentage points of its market share, which now stands at 16 percent, while Roche and PacBio shares held pretty much steady, at 10 and 3 percent, respectively (see charts).



Source: Mizuho Securities and GenomeWeb survey. Number of respondents = 103.



Source: Mizuho Securities and GenomeWeb survey.

Almost half of sixty-two participants who are considering adding a next-gen sequencing instrument this year said they would opt for Oxford Nanopore Technologies' Minlon, and 45 percent for a Gridlon, demonstrating users' considerable interest in the first nanopore sequencing platform to reach the market. Oxford Nano started an early access program for the Minlon late last year but has not said when it will commercialize the Gridlon. In 2012, about a quarter of respondents were considering purchasing either of the Oxford Nano systems, which were not available at that time (see chart).

About 40 percent of respondents said they were considering purchasing a MiSeq, and 40 percent a HiSeq, a slight increase over last year, when 35 percent were thinking about a MiSeq, and 33 percent about a HiSeq.

The Ion Proton is the top choice for 21 percent of respondents, a marked decline from last year, when 35 percent were considering the platform.

About 15 percent are considering the PacBio RS, compared to last year, when only 1 percent were doing so, showing that users have gained more confidence in the platform and its applications.

Thirteen percent are considering the MiSeqDx, which was not available last year, and 13 percent the Qiagen GeneReader, which the company plans to commercialize this year. One participant each said they are thinking about getting a PGM, a SOLiD, or a GnuBio system, which is also expected to be released this year.

turnaround time required for clinical samples. We also want the ability to combine HiSeq and MiSeq data, to save time and cost, and ultimately to provide the best service to our customers."

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# **EXHIBIT 2**



US012151241B2

(12) **United States Patent**  
**Williamson et al.**

(10) **Patent No.: US 12,151,241 B2**  
(45) **Date of Patent: Nov. 26, 2024**

(54) **SYSTEMS, METHODS, AND APPARATUSES  
TO IMAGE A SAMPLE FOR BIOLOGICAL  
OR CHEMICAL ANALYSIS**

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(71) Applicant: **ILLUMINA, INC.**, San Diego, CA  
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**Drew Verkade**, Carlsbad, CA (US);  
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(73) Assignee: **ILLUMINA, INC.**, San Diego, CA  
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(\*) Notice: Subject to any disclaimer, the term of this  
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(21) Appl. No.: **18/586,010**

(22) Filed: **Feb. 23, 2024**

(65) **Prior Publication Data**

US 2024/0189817 A1 Jun. 13, 2024

**Related U.S. Application Data**

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May 8, 2023, now Pat. No. 11,938,479, which is a  
(Continued)

(51) **Int. Cl.**  
**B01L 3/00** (2006.01)  
**B01L 7/00** (2006.01)  
(Continued)

(52) **U.S. Cl.**  
CPC ..... **B01L 3/502715** (2013.01); **B01L 9/527**  
(2013.01); **G01N 21/05** (2013.01);  
(Continued)

(58) **Field of Classification Search**  
CPC ..... B01L 3/502715; B01L 9/527; B01L  
3/50273; B01L 7/52; B01L 2200/025;  
(Continued)

*Primary Examiner* — Jill A Warden

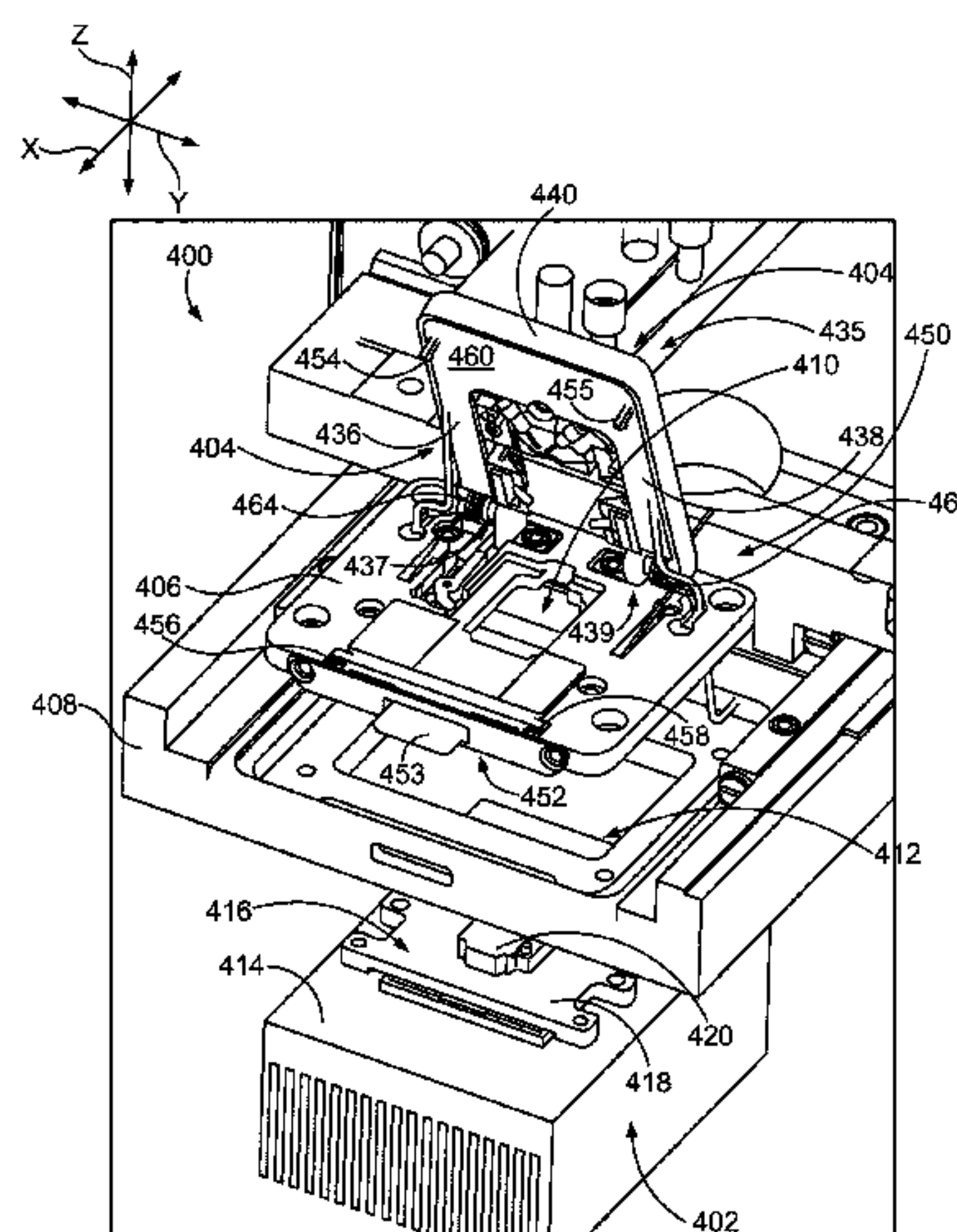
*Assistant Examiner* — Jacqueline Brazin

(74) *Attorney, Agent, or Firm* — MARSHALL,  
GERSTEIN & BORUN LLP

(57) **ABSTRACT**

A fluidic device holder configured to orient a fluidic device. The device holder includes a support structure configured to receive a fluidic device. The support structure includes a base surface that faces in a direction along the Z-axis and is configured to have the fluidic device positioned thereon. The device holder also includes a plurality of reference surfaces facing in respective directions along an XY-plane. The device holder also includes an alignment assembly having an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end away from and toward the reference surfaces. The locator arm is configured to hold the fluidic device against the reference surfaces when the locator arm is in the biased position.

**20 Claims, 39 Drawing Sheets**





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## Related U.S. Application Data

continuation of application No. 17/714,129, filed on Apr. 5, 2022, now Pat. No. 11,697,116, which is a continuation of application No. 16/255,546, filed on Jan. 23, 2019, now Pat. No. 11,559,805, which is a division of application No. 14/550,956, filed on Nov. 22, 2014, now Pat. No. 10,220,386, which is a continuation of application No. 13/273,666, filed on Oct. 14, 2011, now Pat. No. 8,951,781.

- (60) Provisional application No. 61/438,530, filed on Feb. 1, 2011, provisional application No. 61/438,567, filed on Feb. 1, 2011, provisional application No. 61/438,486, filed on Feb. 1, 2011, provisional application No. 61/431,439, filed on Jan. 11, 2011, provisional application No. 61/431,440, filed on Jan. 11, 2011, provisional application No. 61/431,425, filed on Jan. 10, 2011, provisional application No. 61/431,429, filed on Jan. 10, 2011.

- (51) **Int. Cl.**  
**B01L 9/00** (2006.01)  
**G01N 21/05** (2006.01)

- (52) **U.S. Cl.**  
CPC ..... *B01L 3/50273* (2013.01); *B01L 7/52* (2013.01); *B01L 2200/025* (2013.01); *B01L 2200/027* (2013.01); *B01L 2200/04* (2013.01); *B01L 2200/0689* (2013.01); *B01L 2300/022* (2013.01); *B01L 2300/041* (2013.01); *B01L 2300/043* (2013.01); *B01L 2300/0816* (2013.01); *B01L 2300/0877* (2013.01); *G01N 2021/058* (2013.01); *Y10T 436/25* (2015.01)

- (58) **Field of Classification Search**  
CPC ..... B01L 2200/027; B01L 2200/04; B01L 2200/0689; B01L 2300/022; B01L 2300/041; B01L 2300/043; B01L 2300/0816; B01L 2300/0877; G01N 21/05; G01N 2021/058; Y10T 436/25  
USPC ..... 422/554  
See application file for complete search history.

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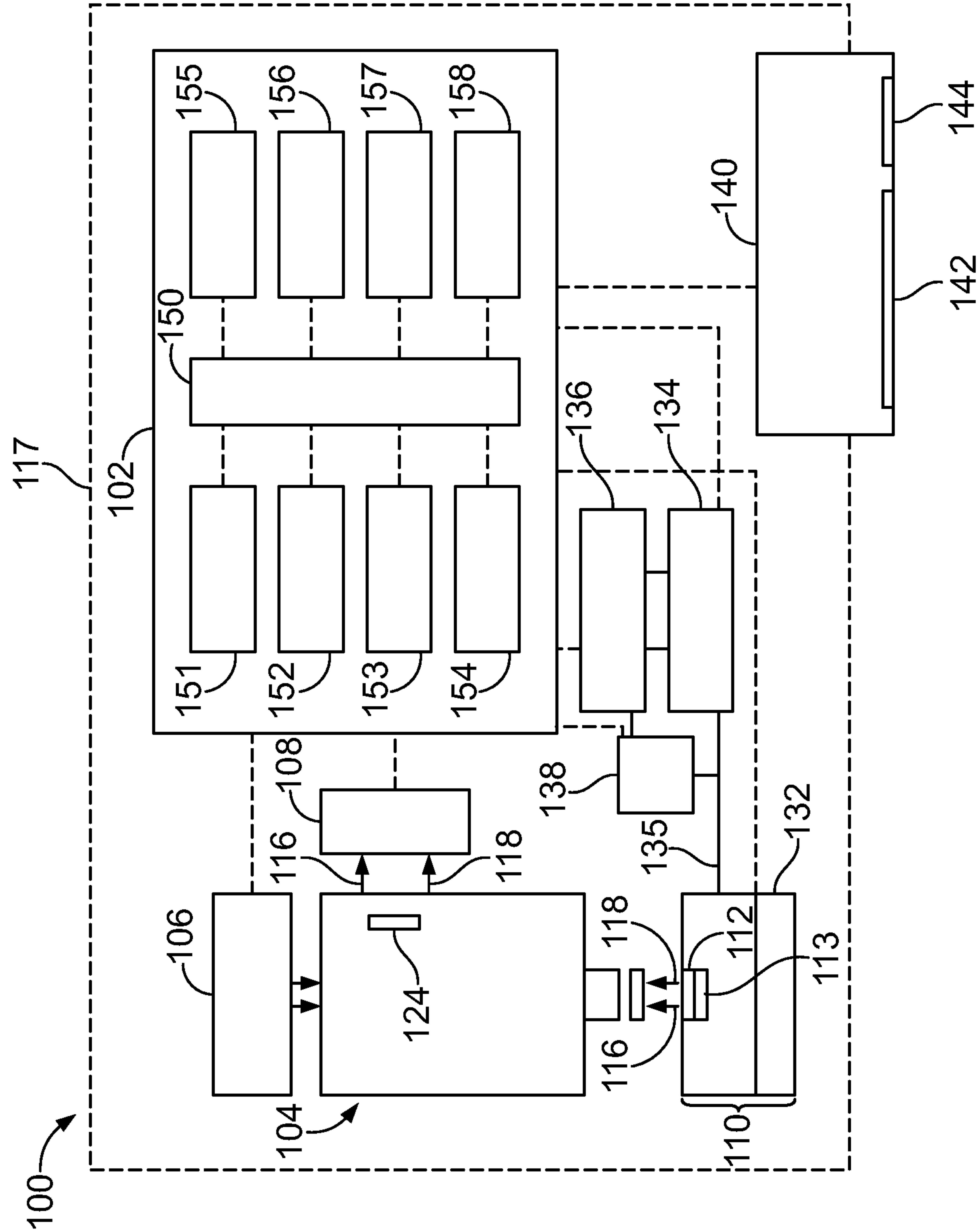


FIG. 1

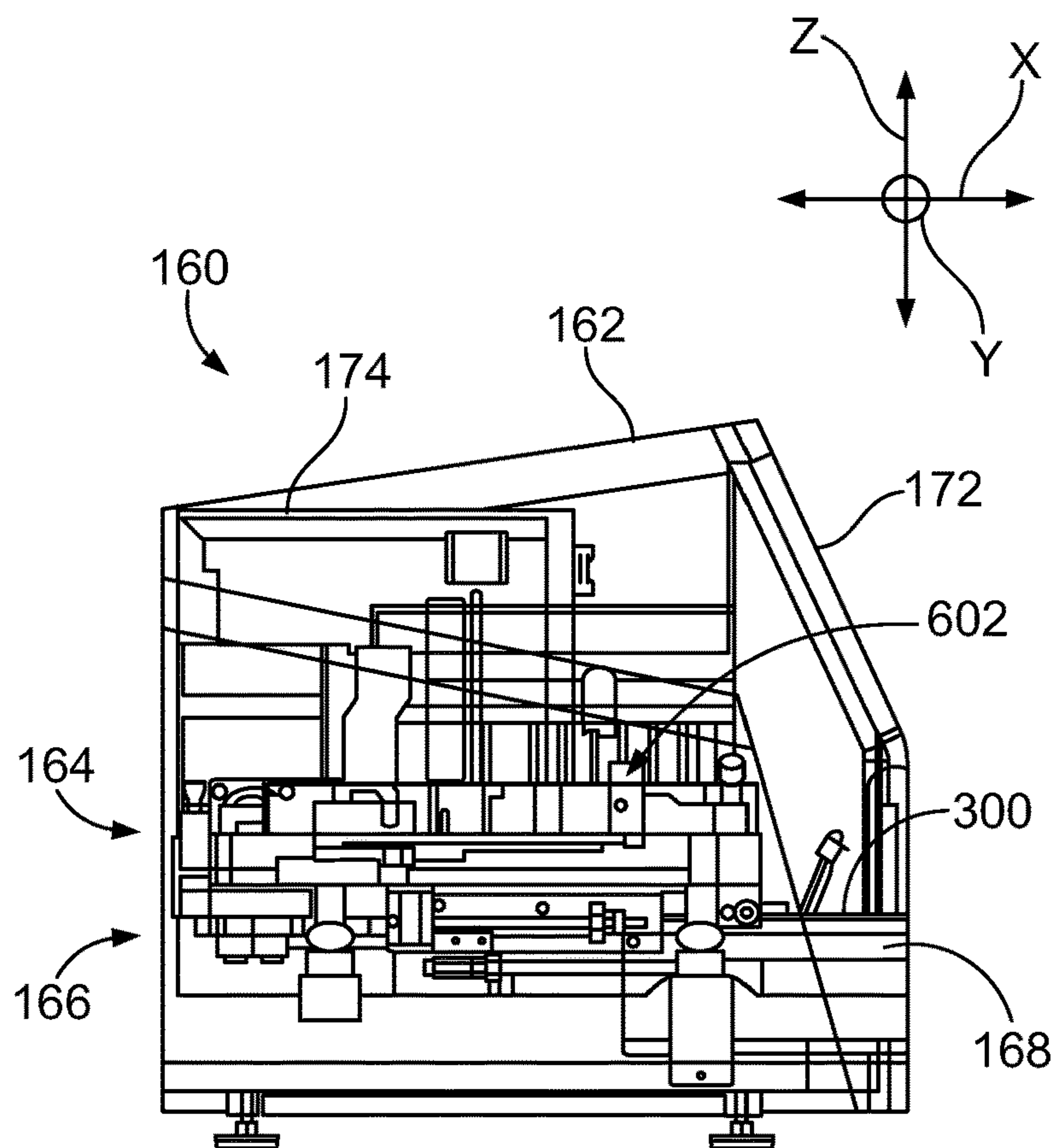


FIG. 2

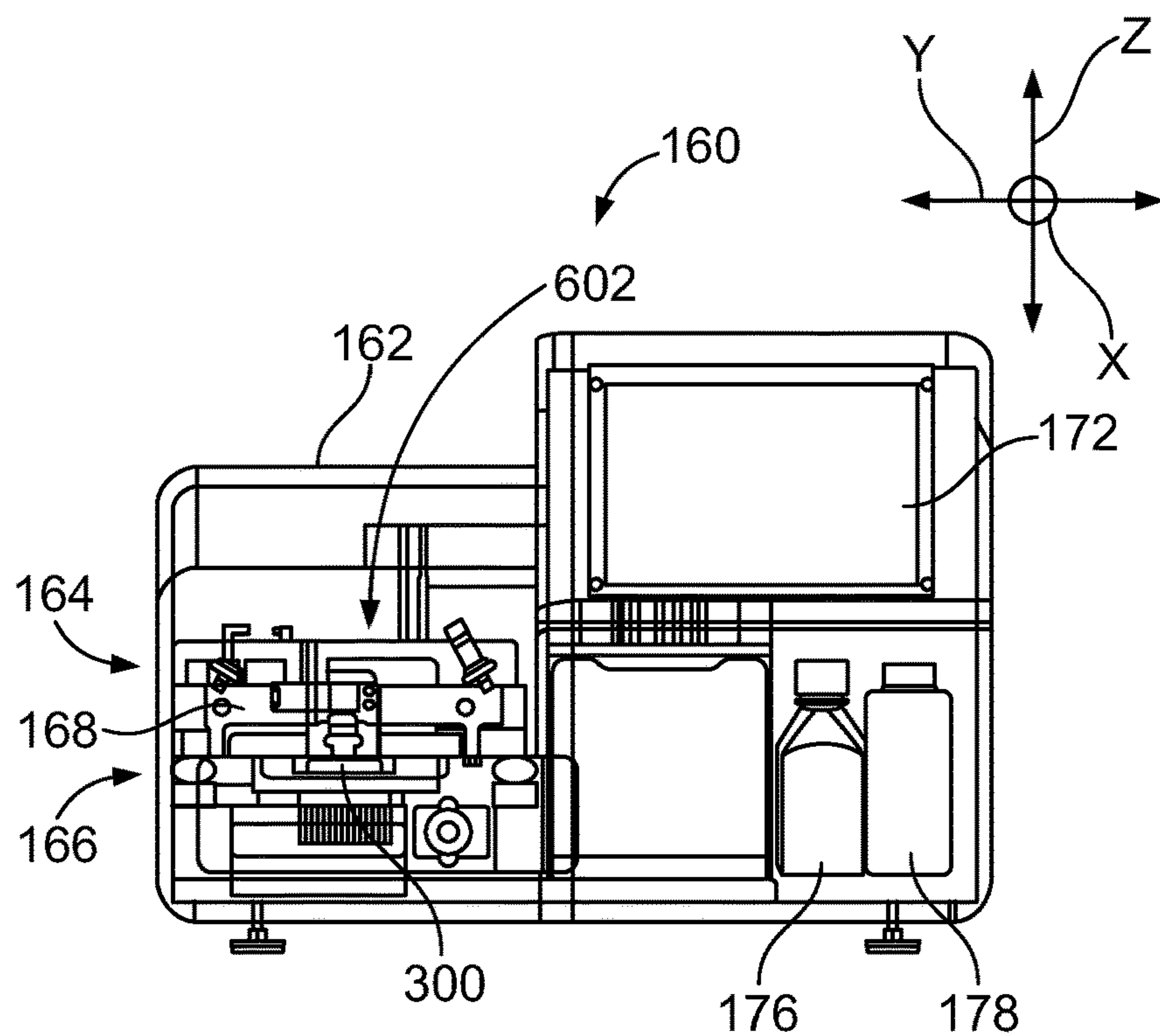


FIG. 3



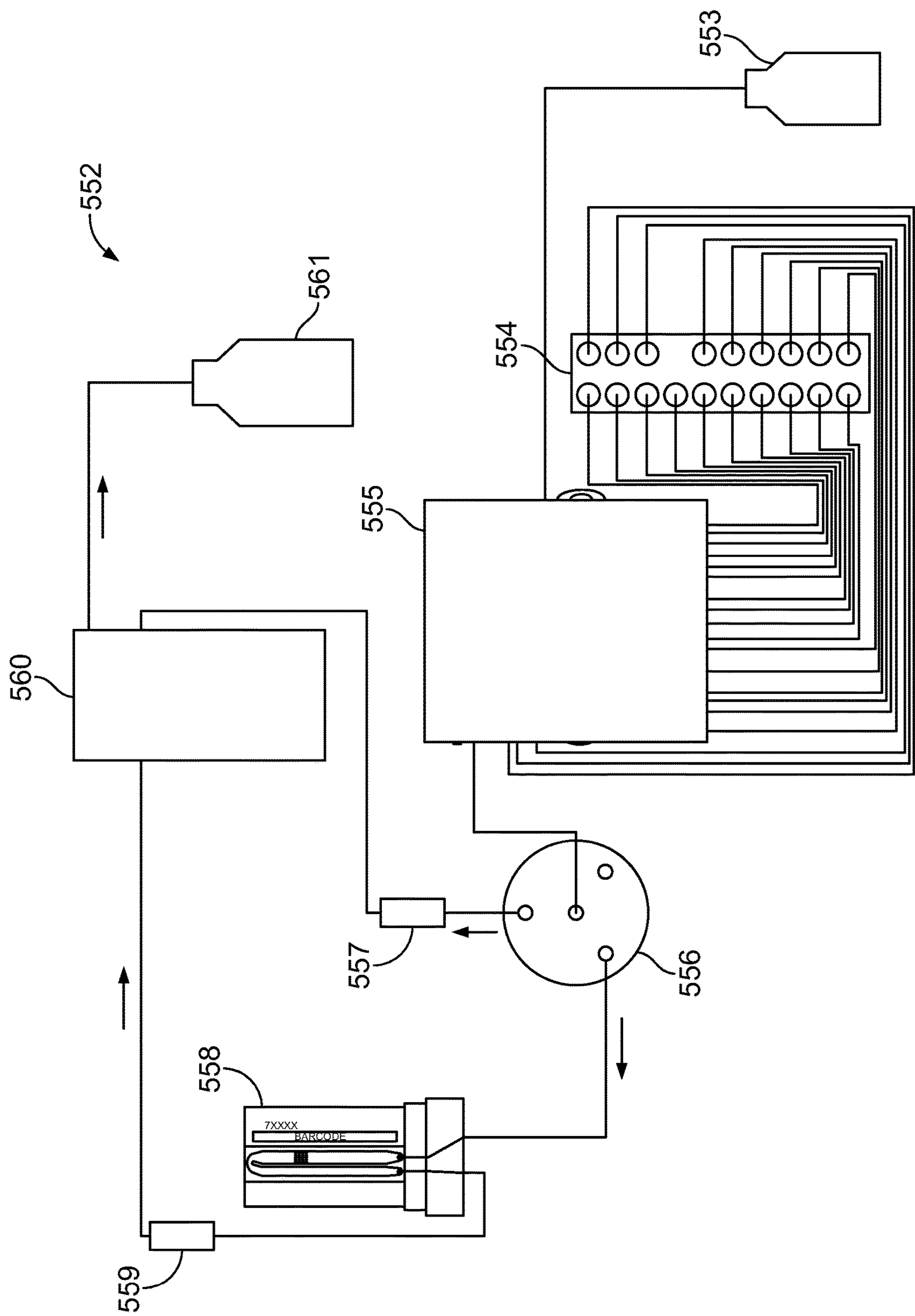
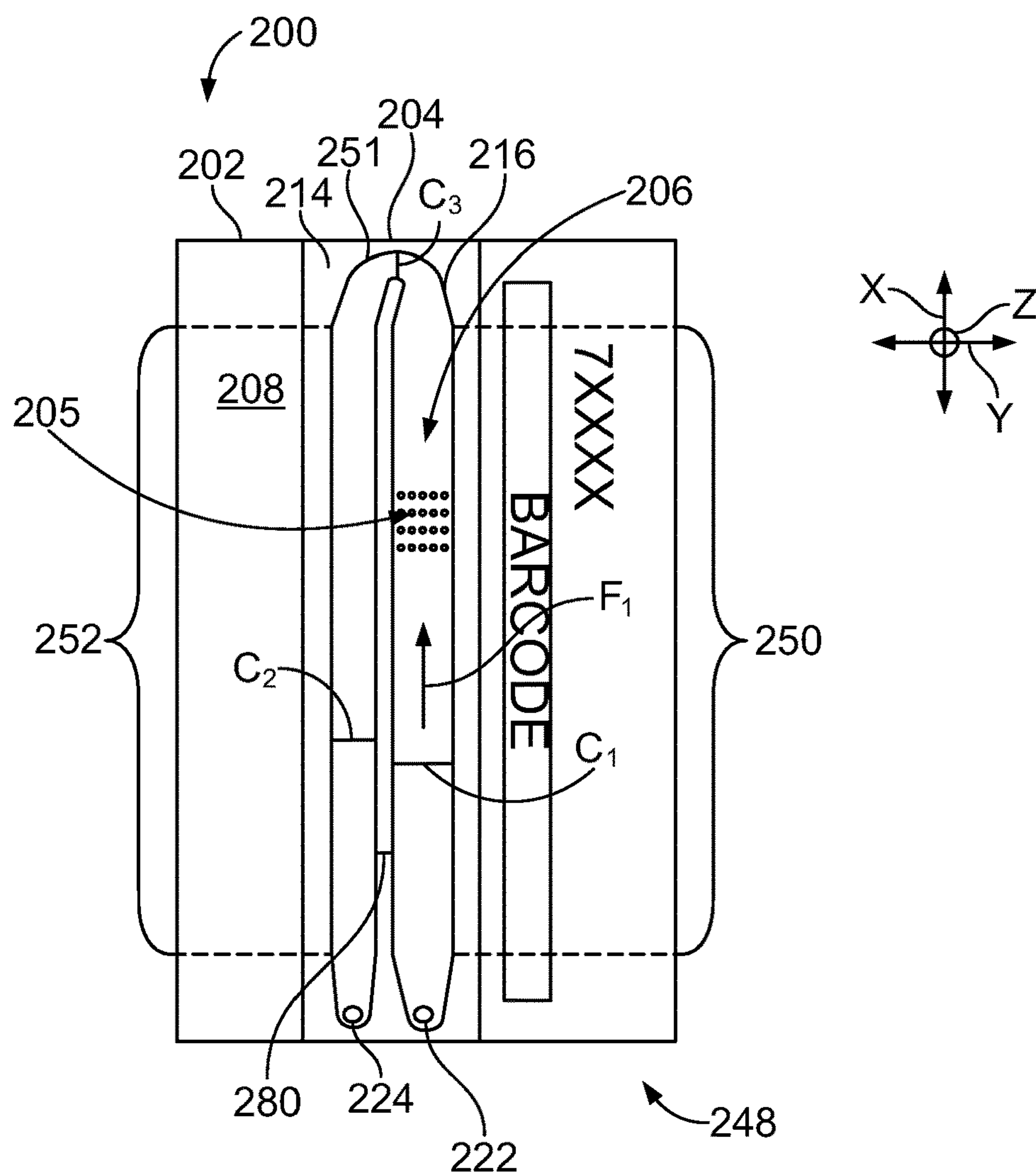
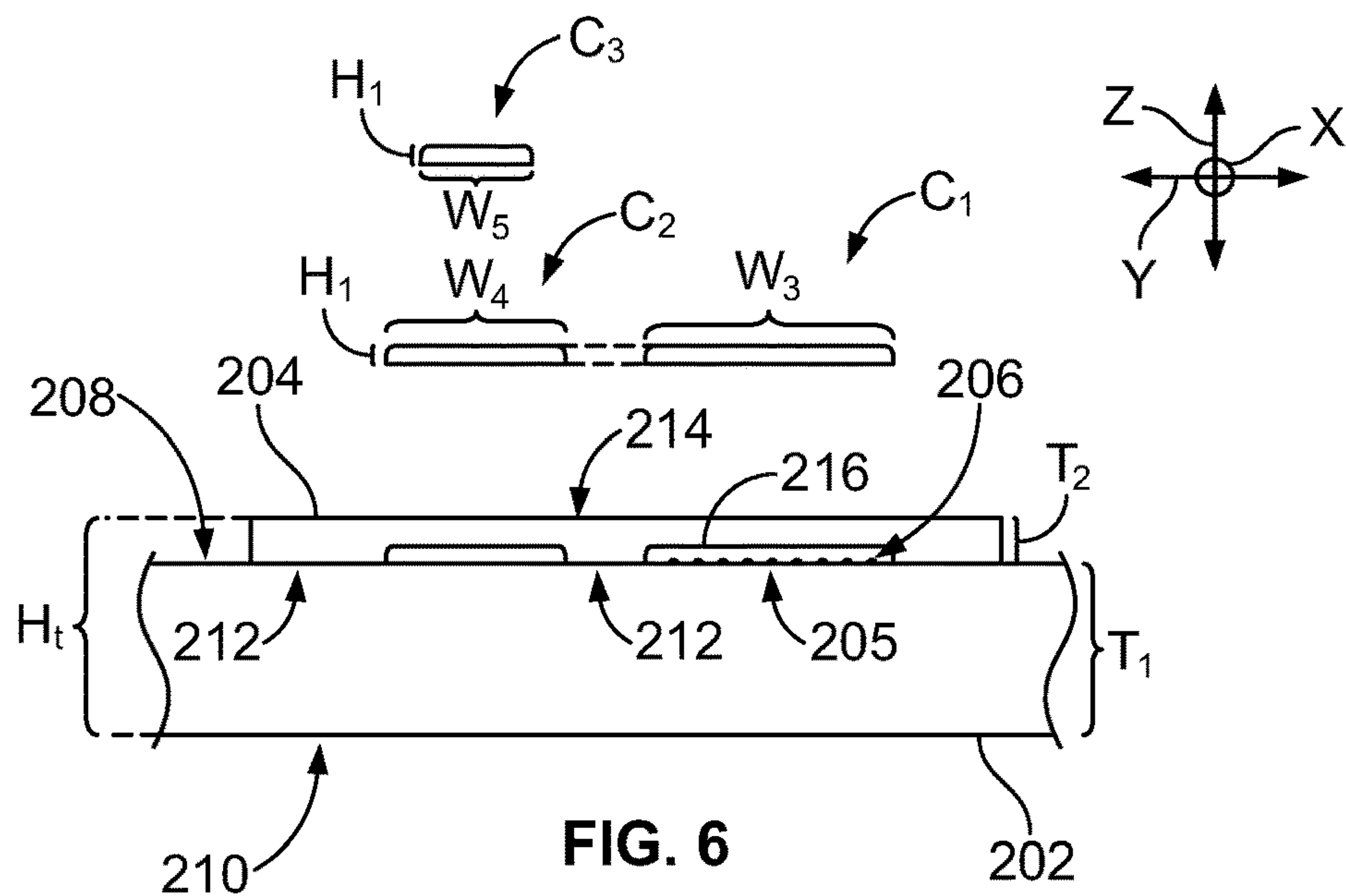


FIG. 4









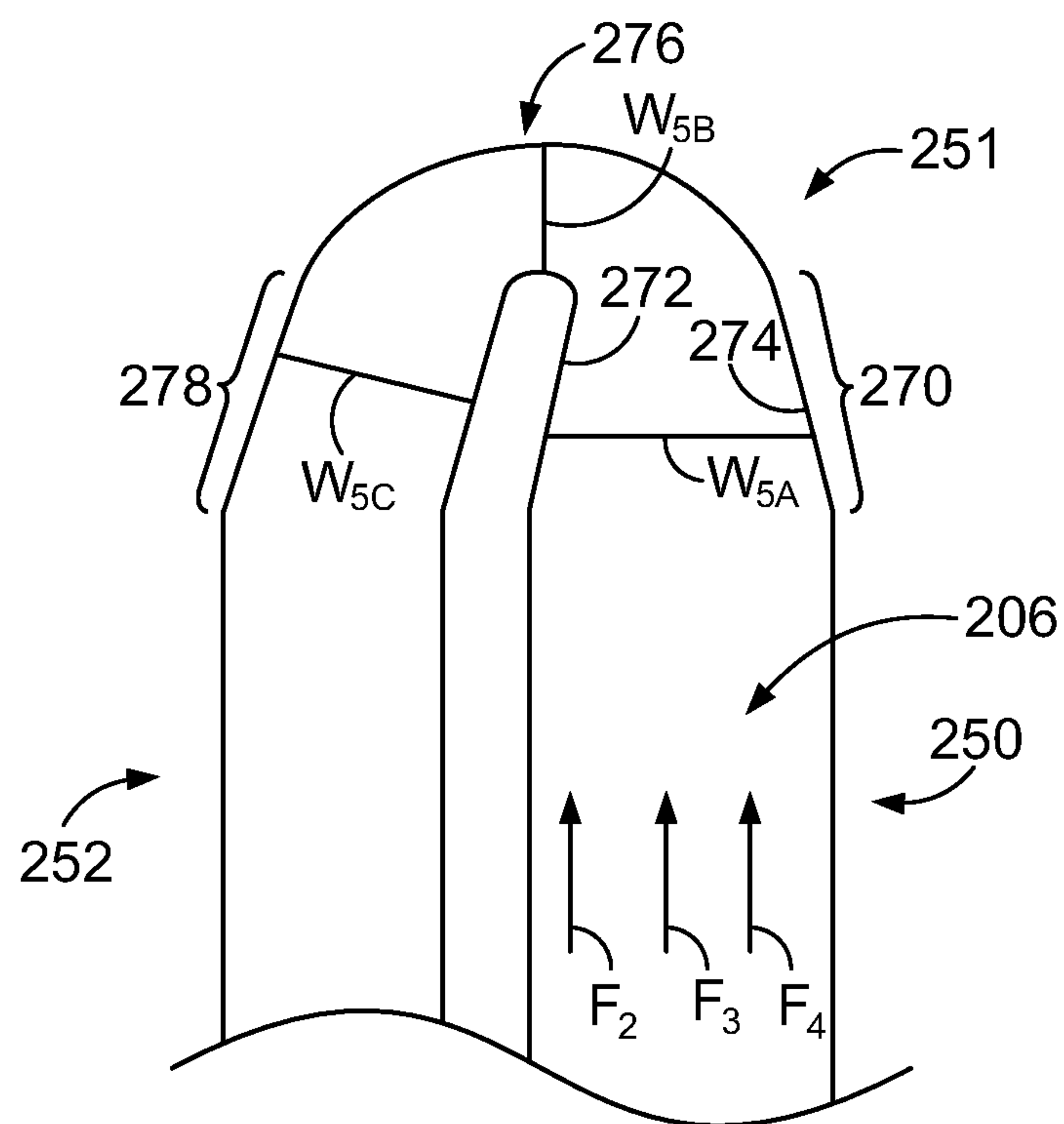


FIG. 8



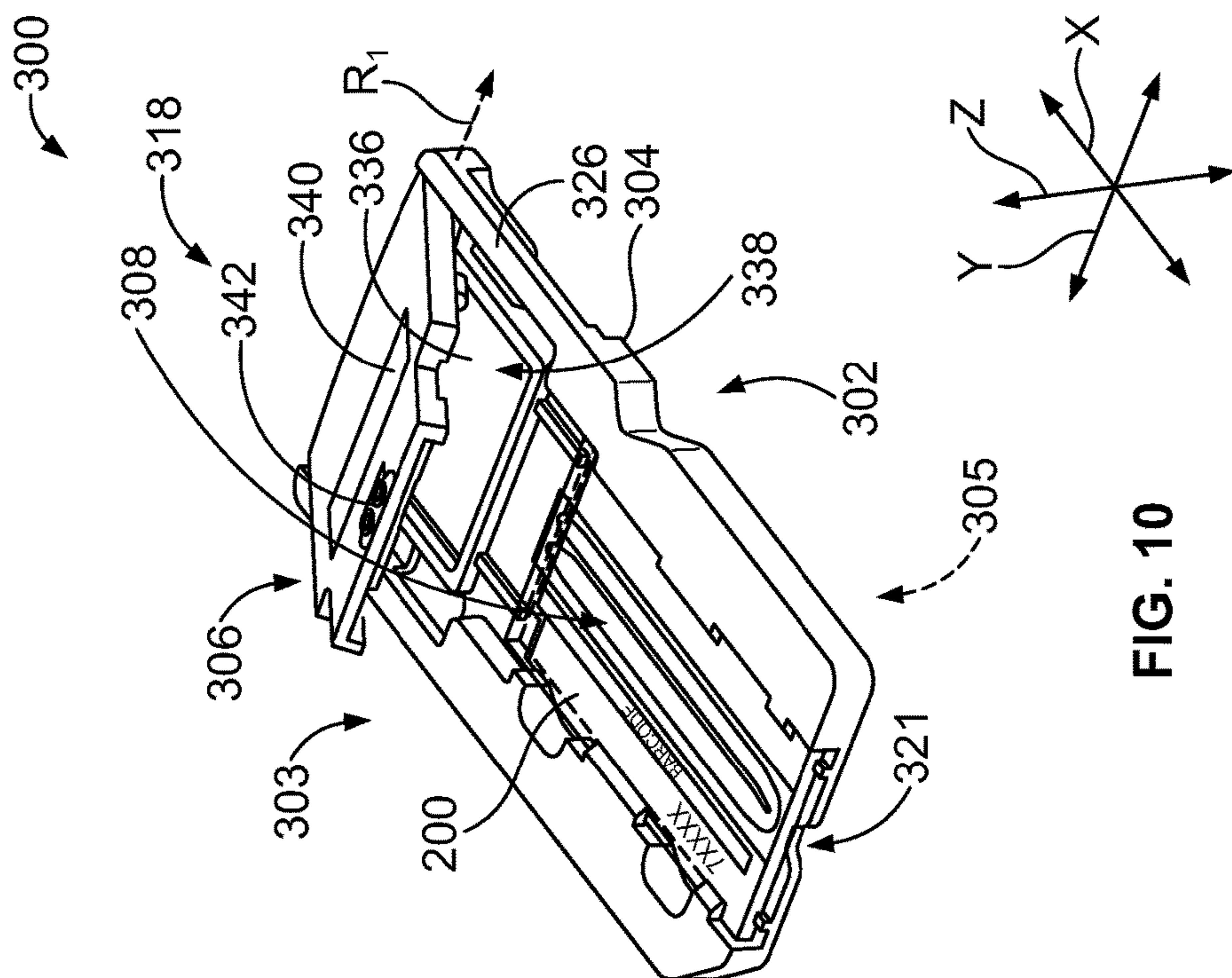


FIG. 10

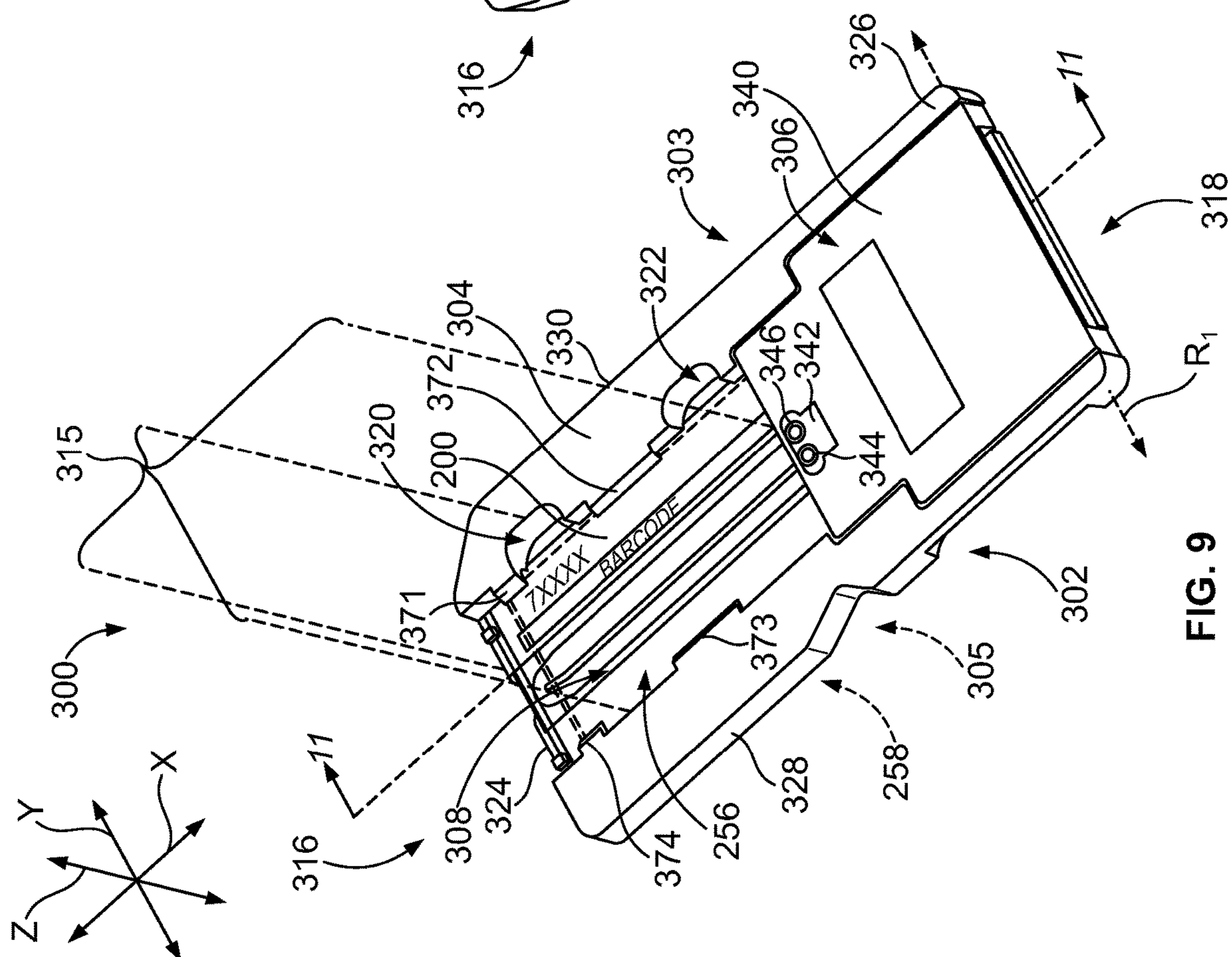


FIG. 9





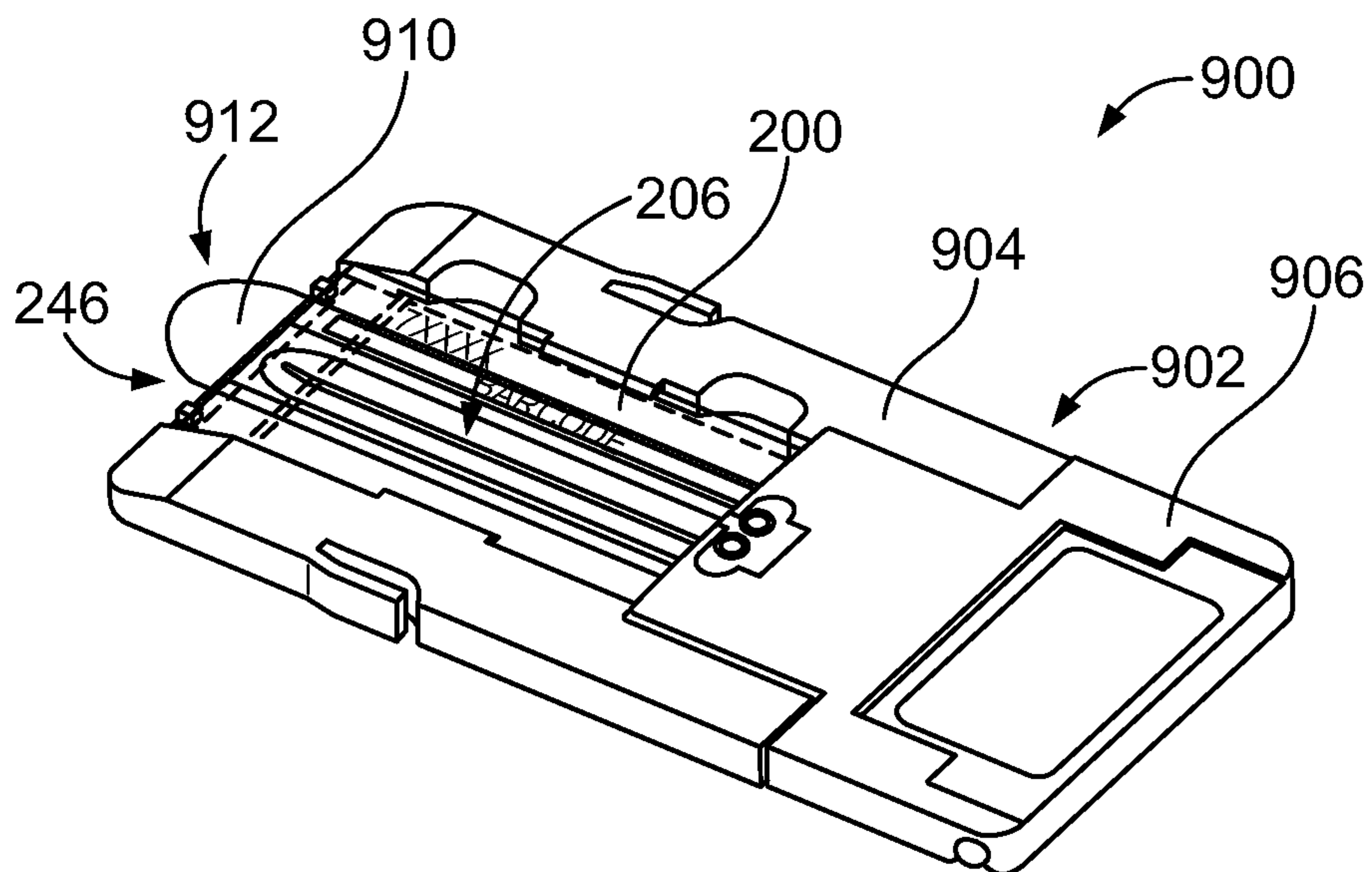


FIG. 12

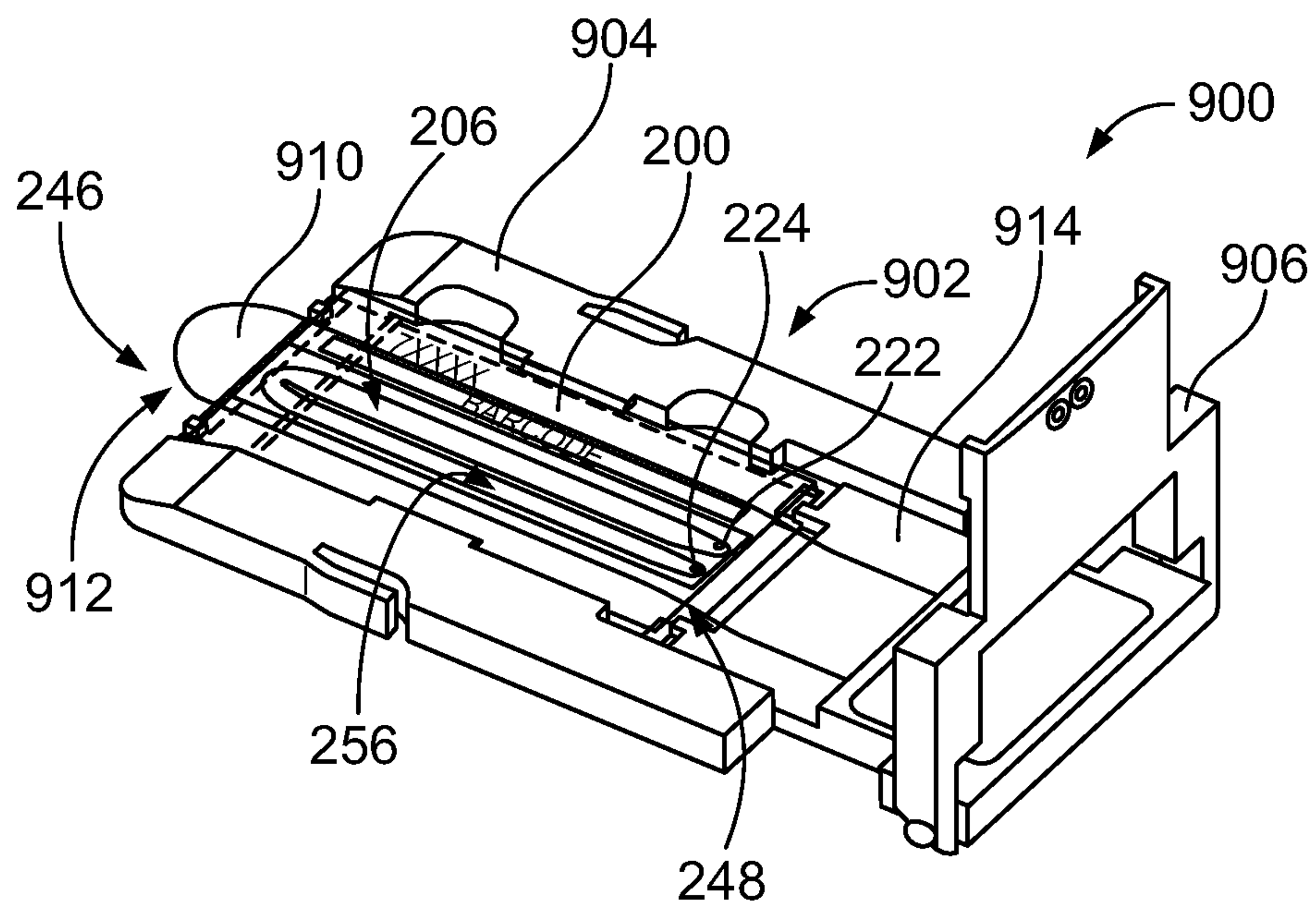


FIG. 13



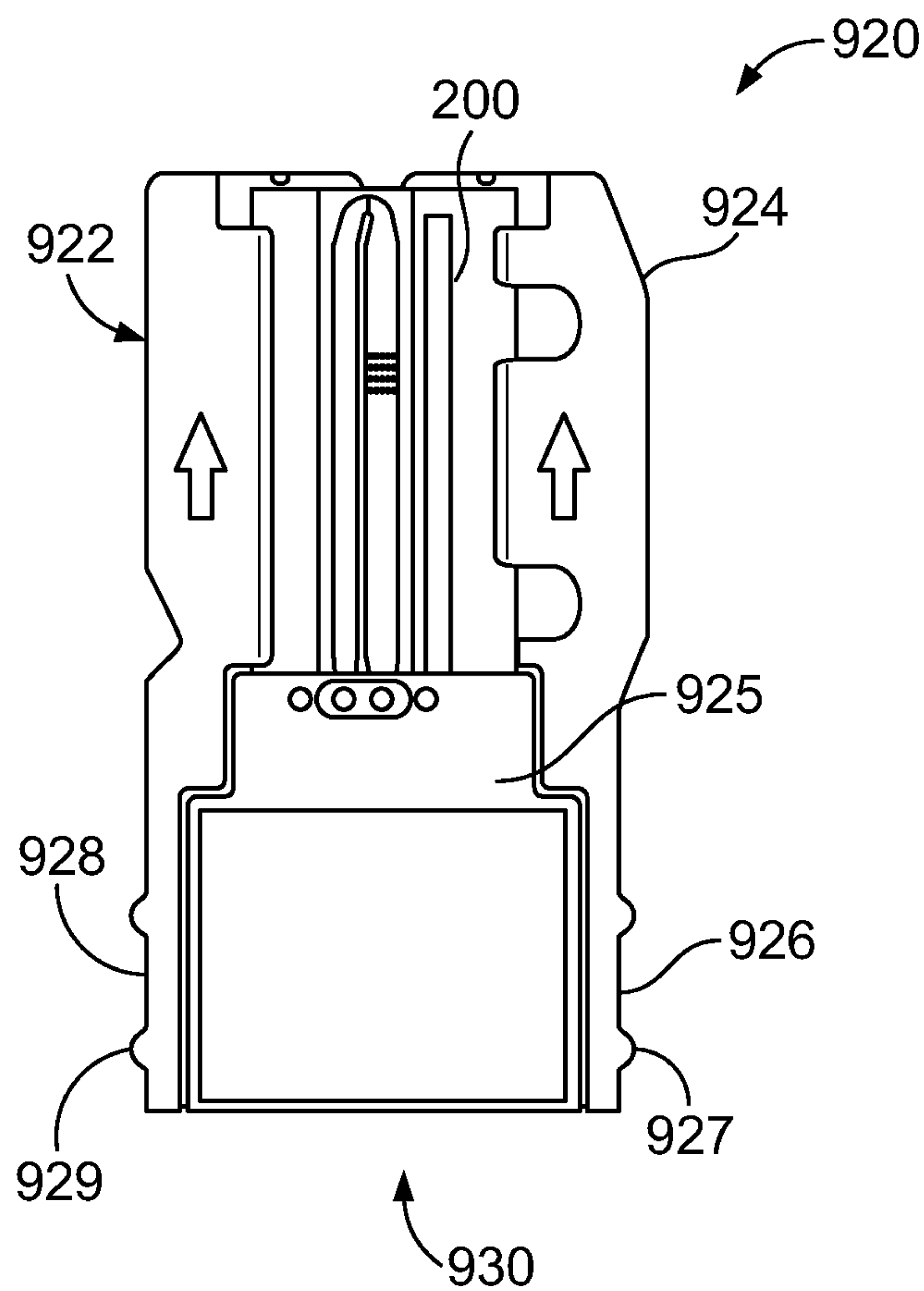


FIG. 14

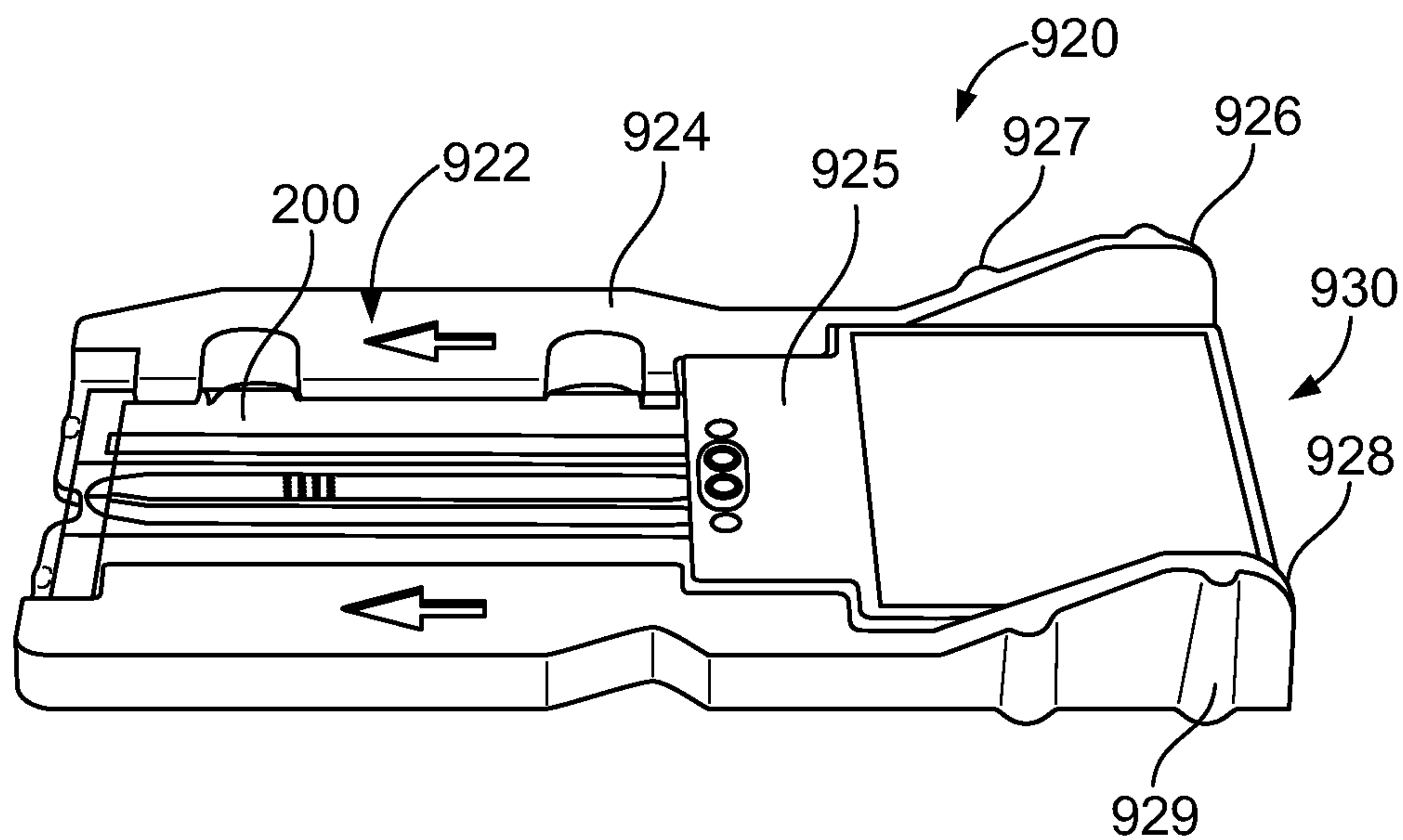


FIG. 15



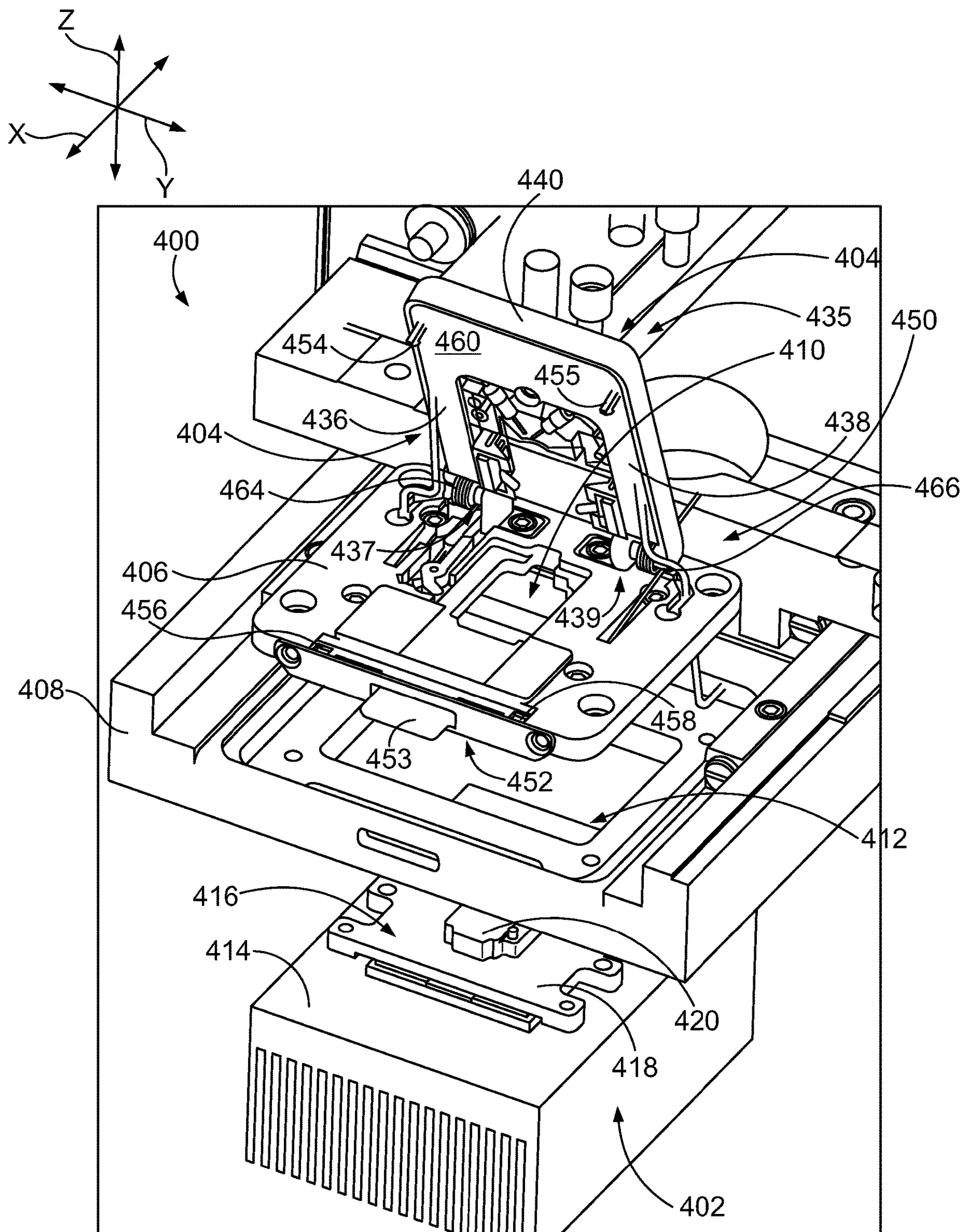


FIG. 16



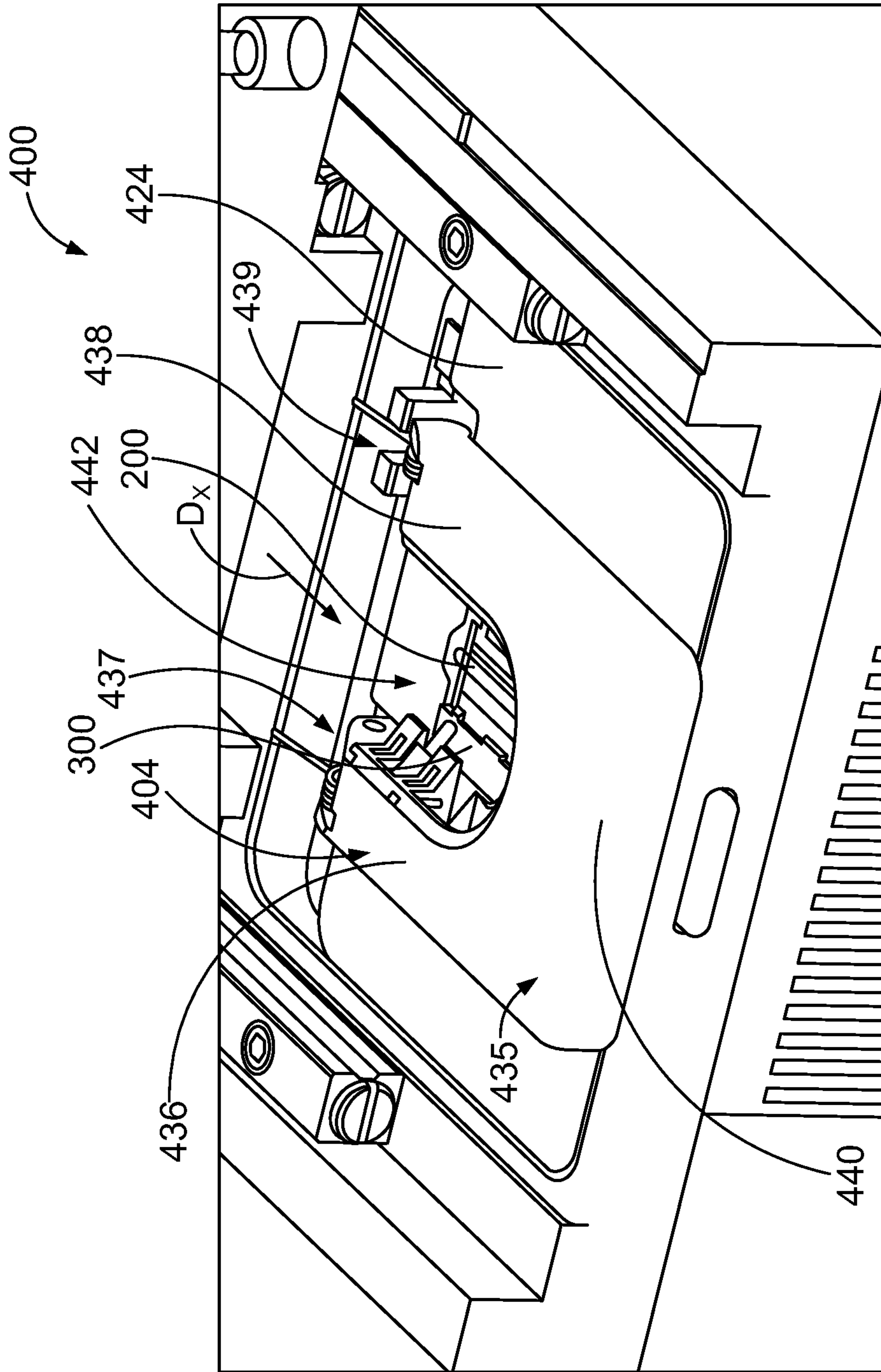


FIG. 17

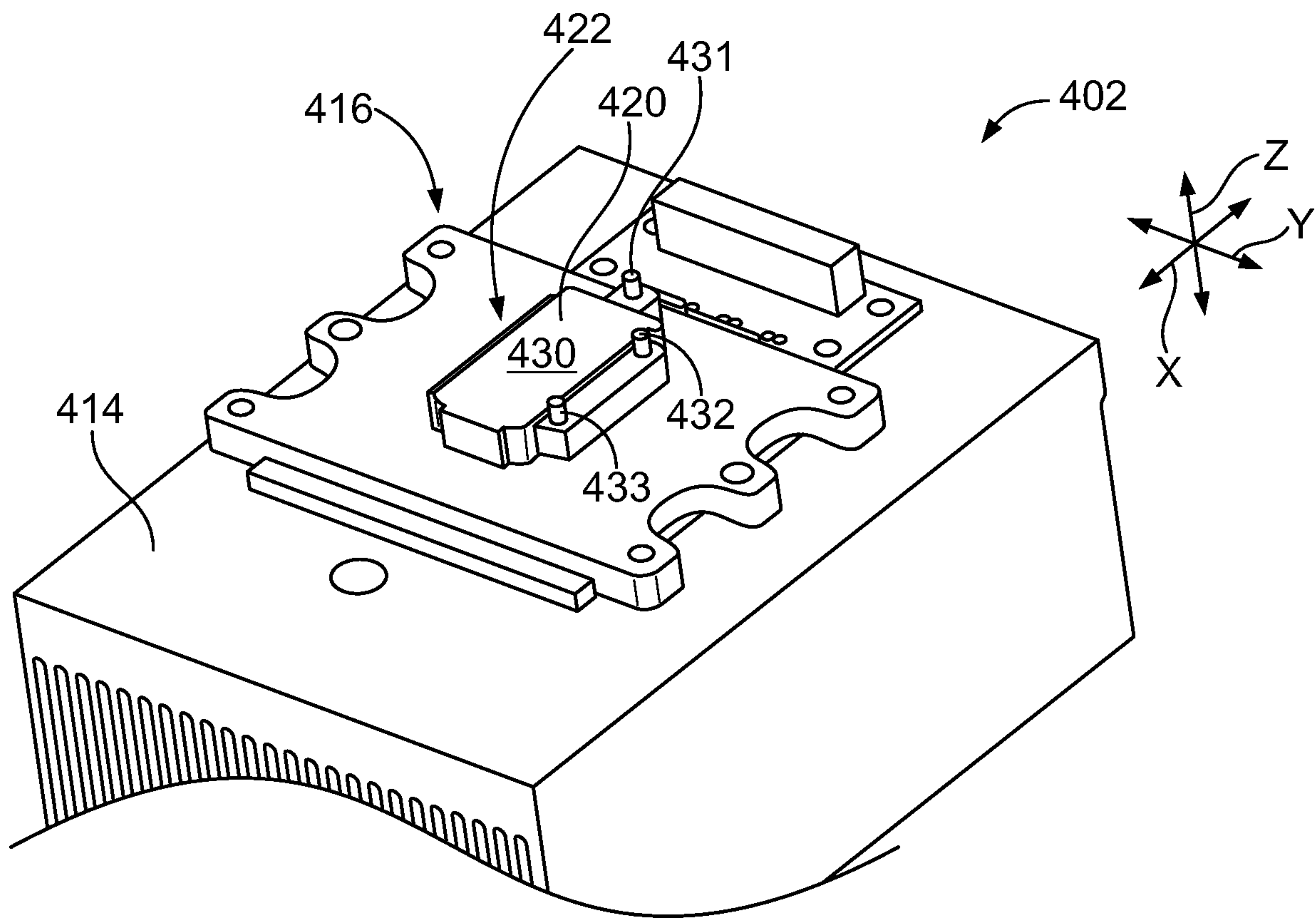


FIG. 18



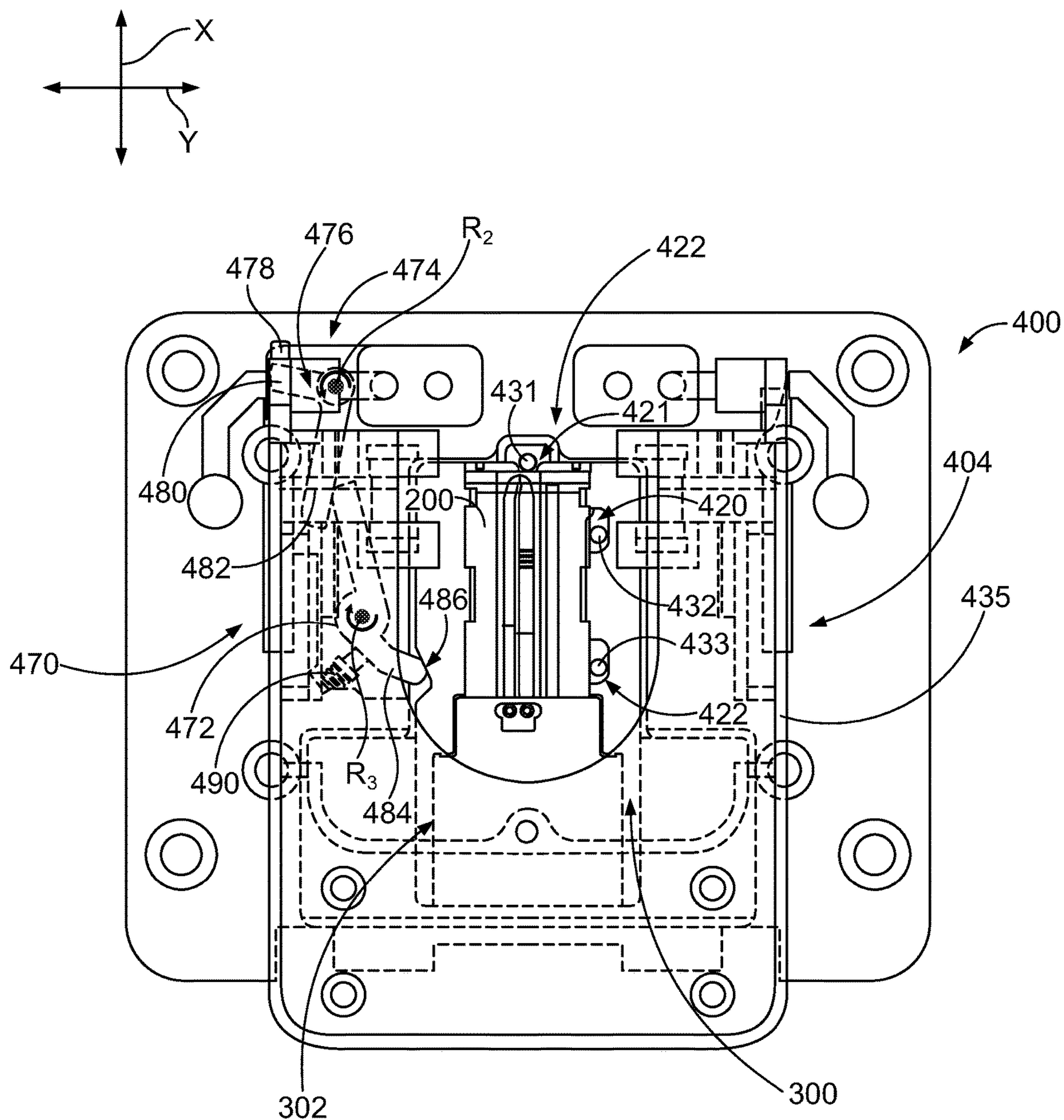


FIG. 19



FIG. 20

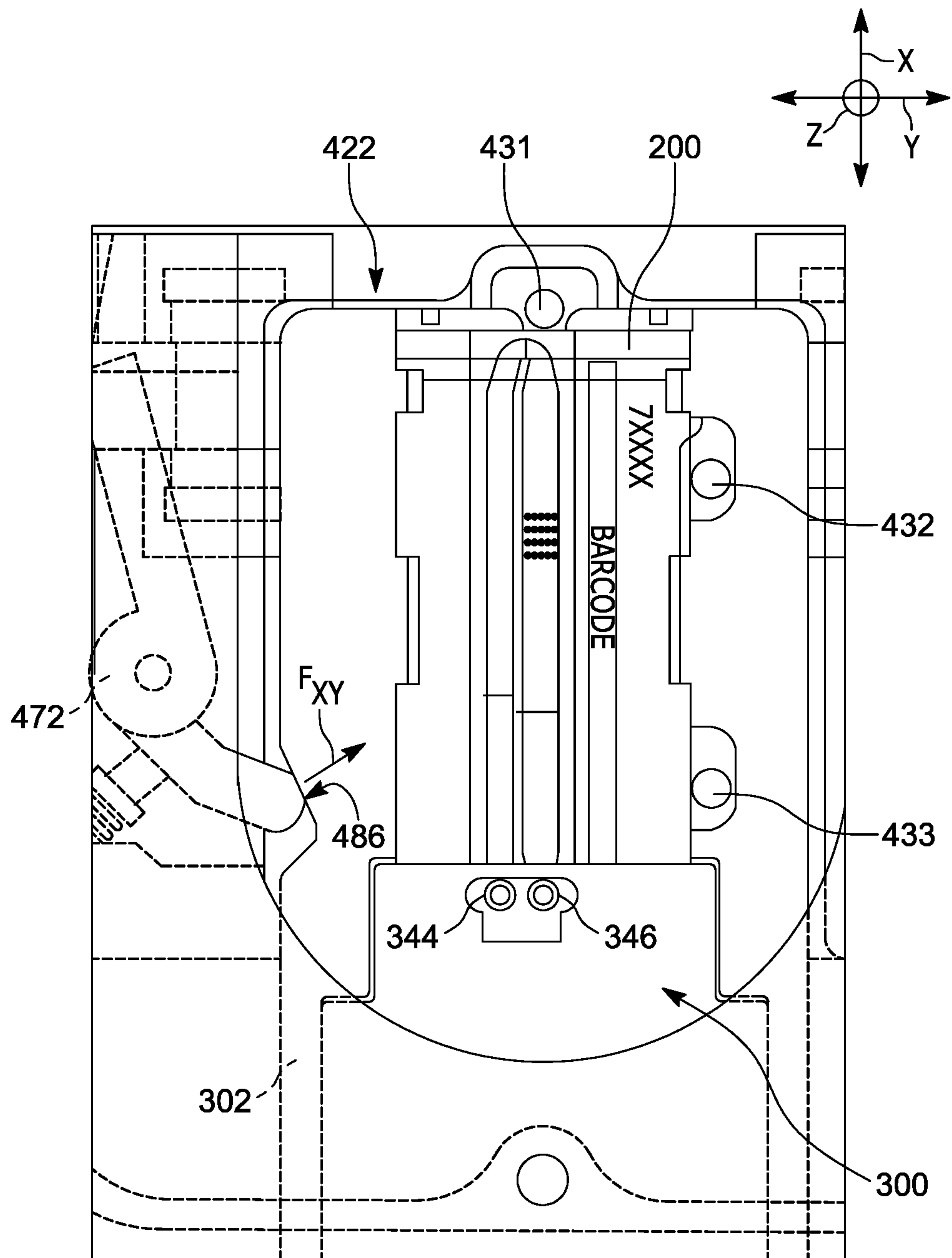


FIG. 21



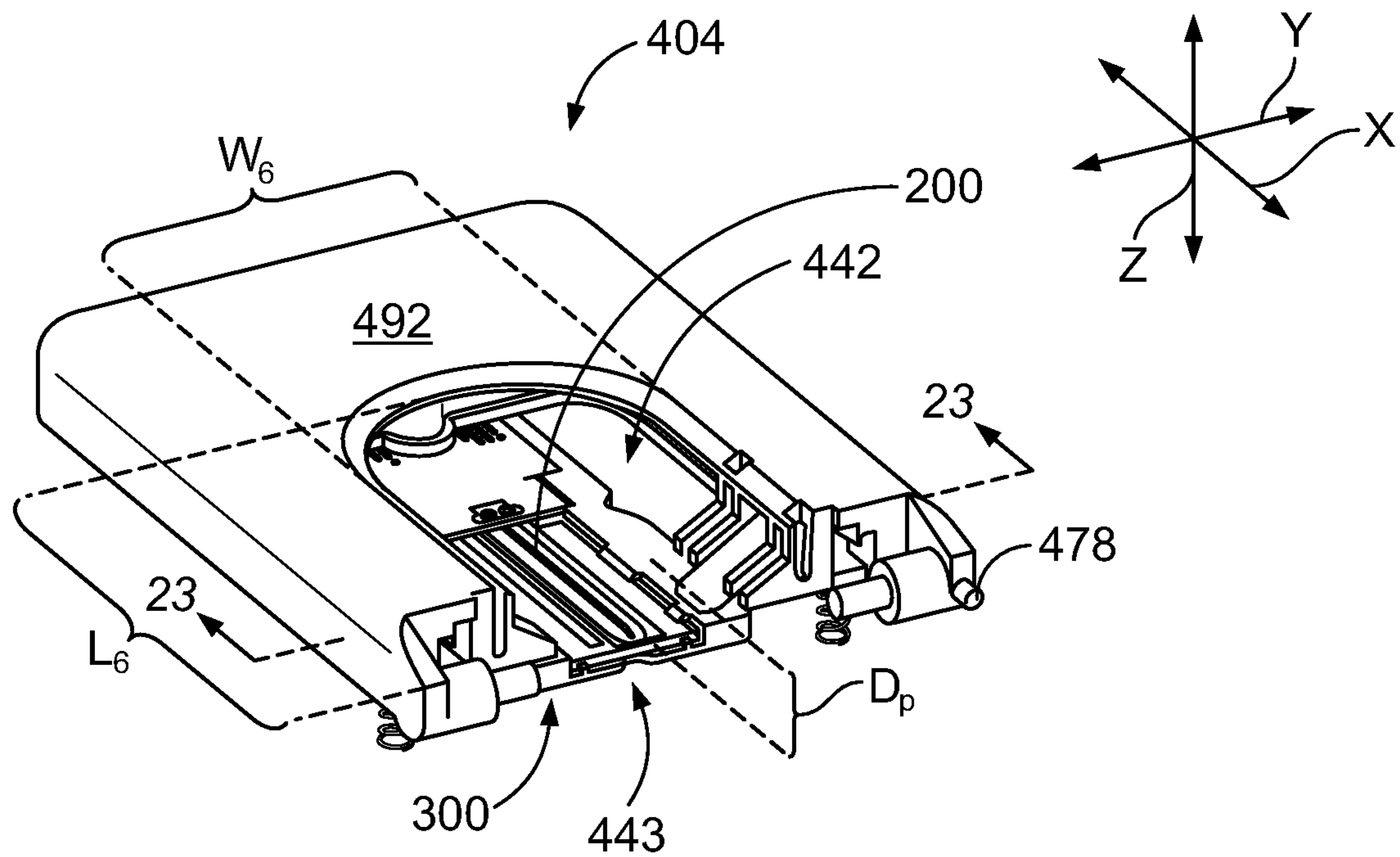


FIG. 22

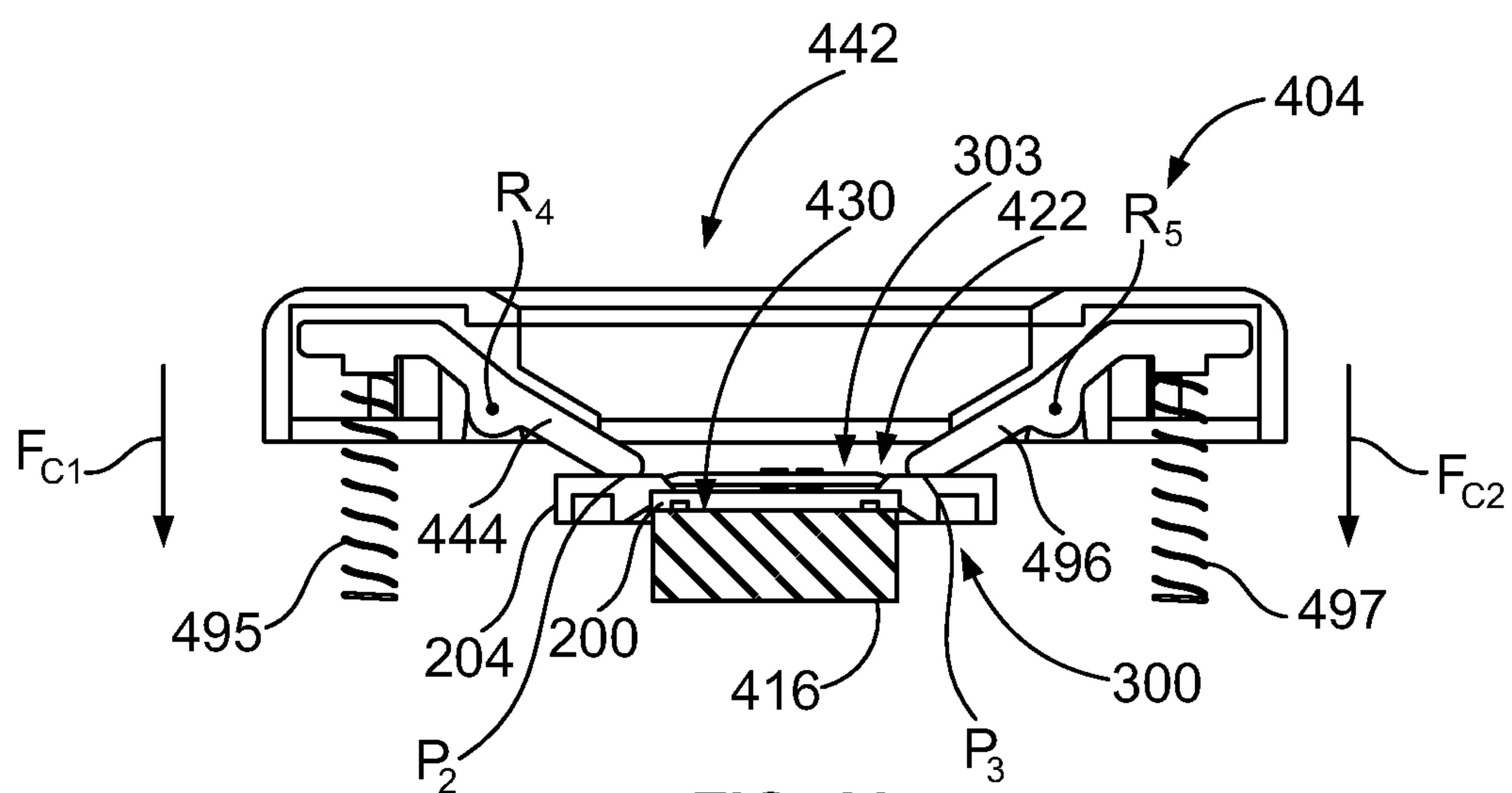


FIG. 23



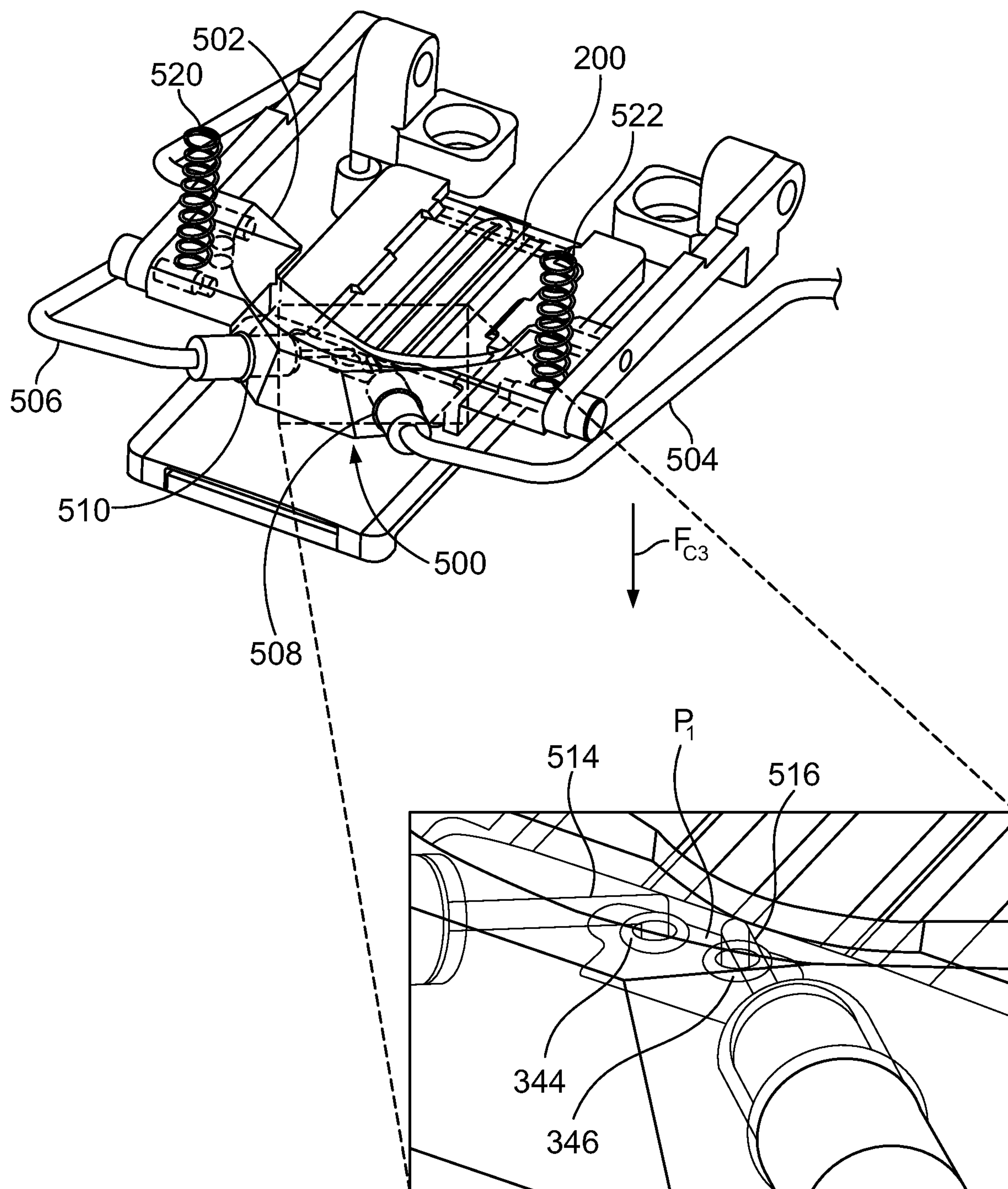


FIG. 24



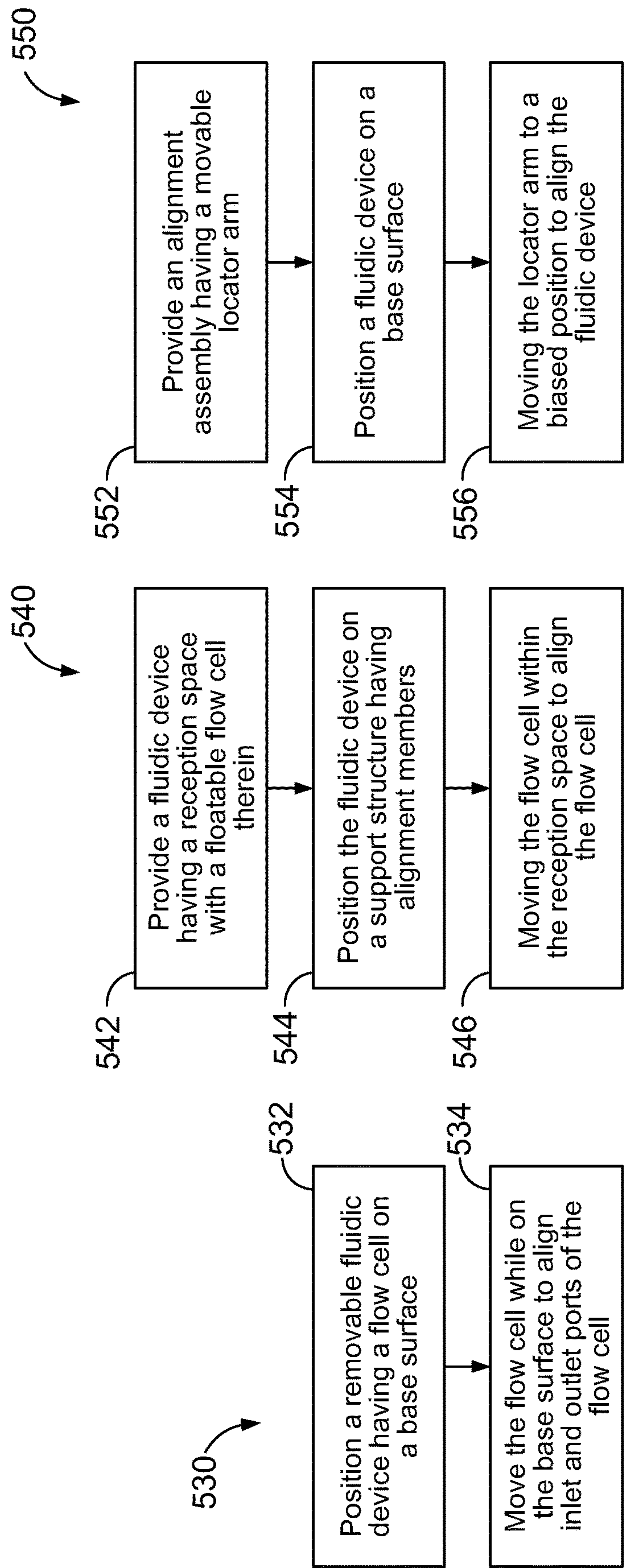


FIG. 25

FIG. 26

FIG. 27

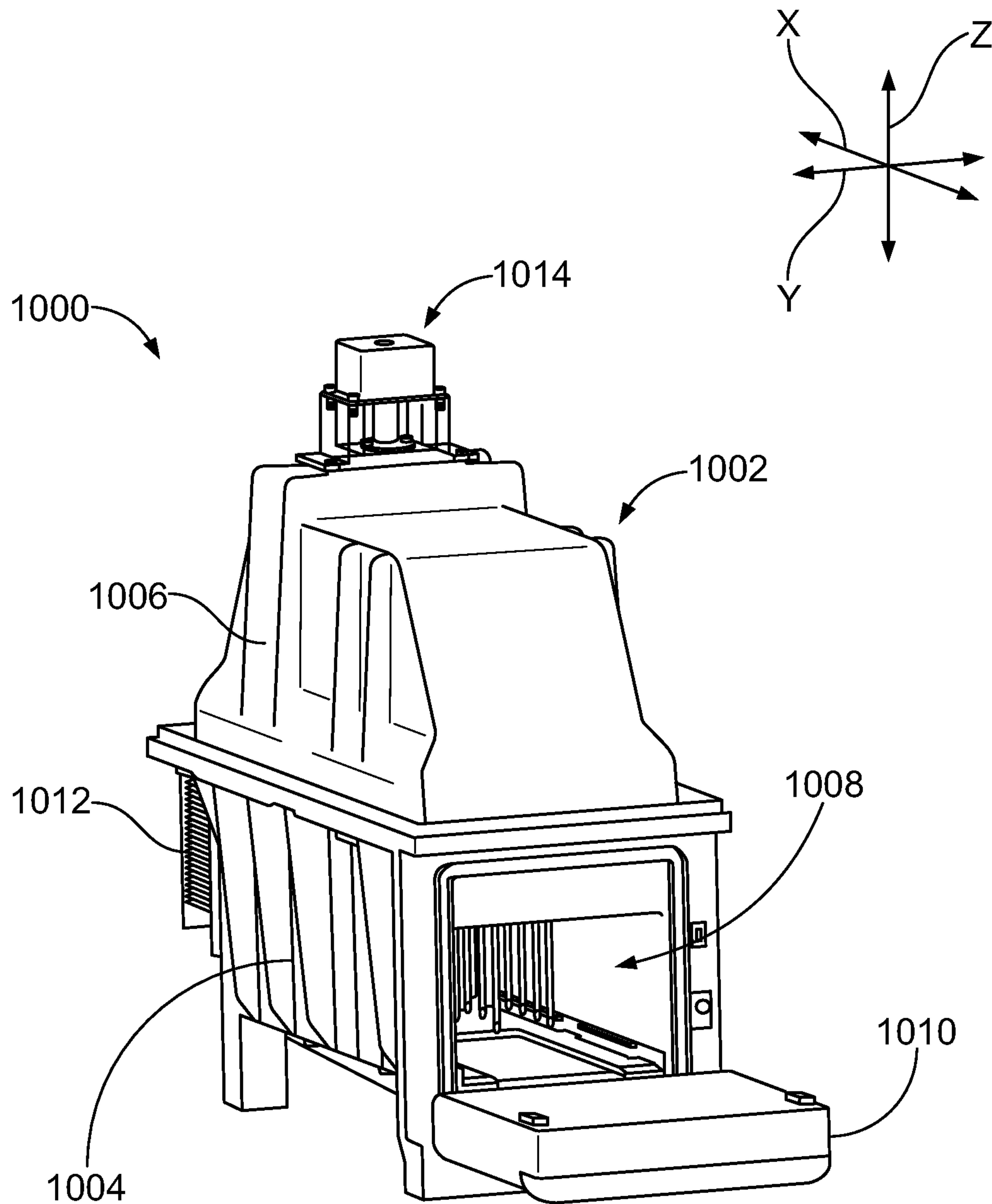


FIG. 28



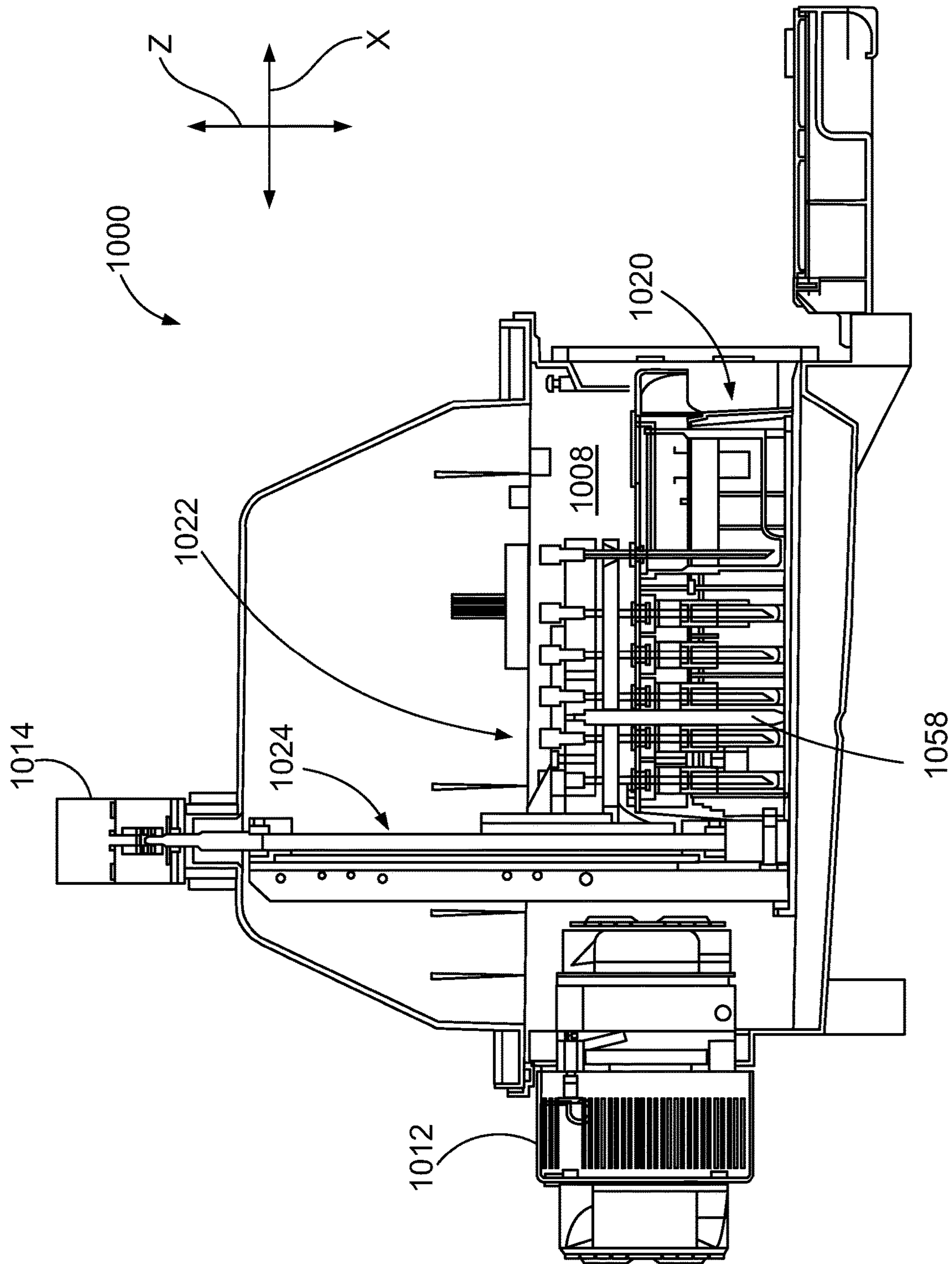


FIG. 29





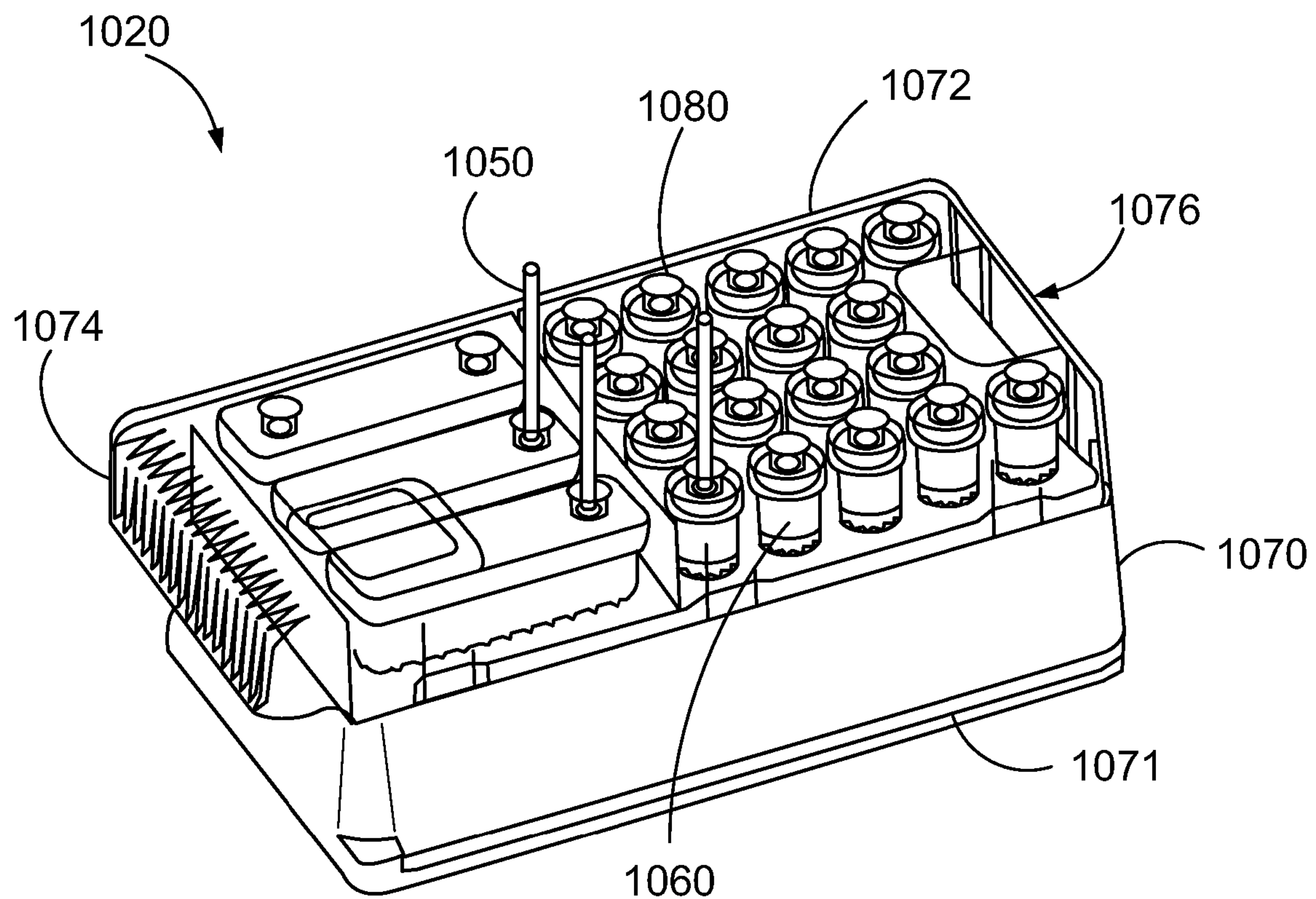


FIG. 31

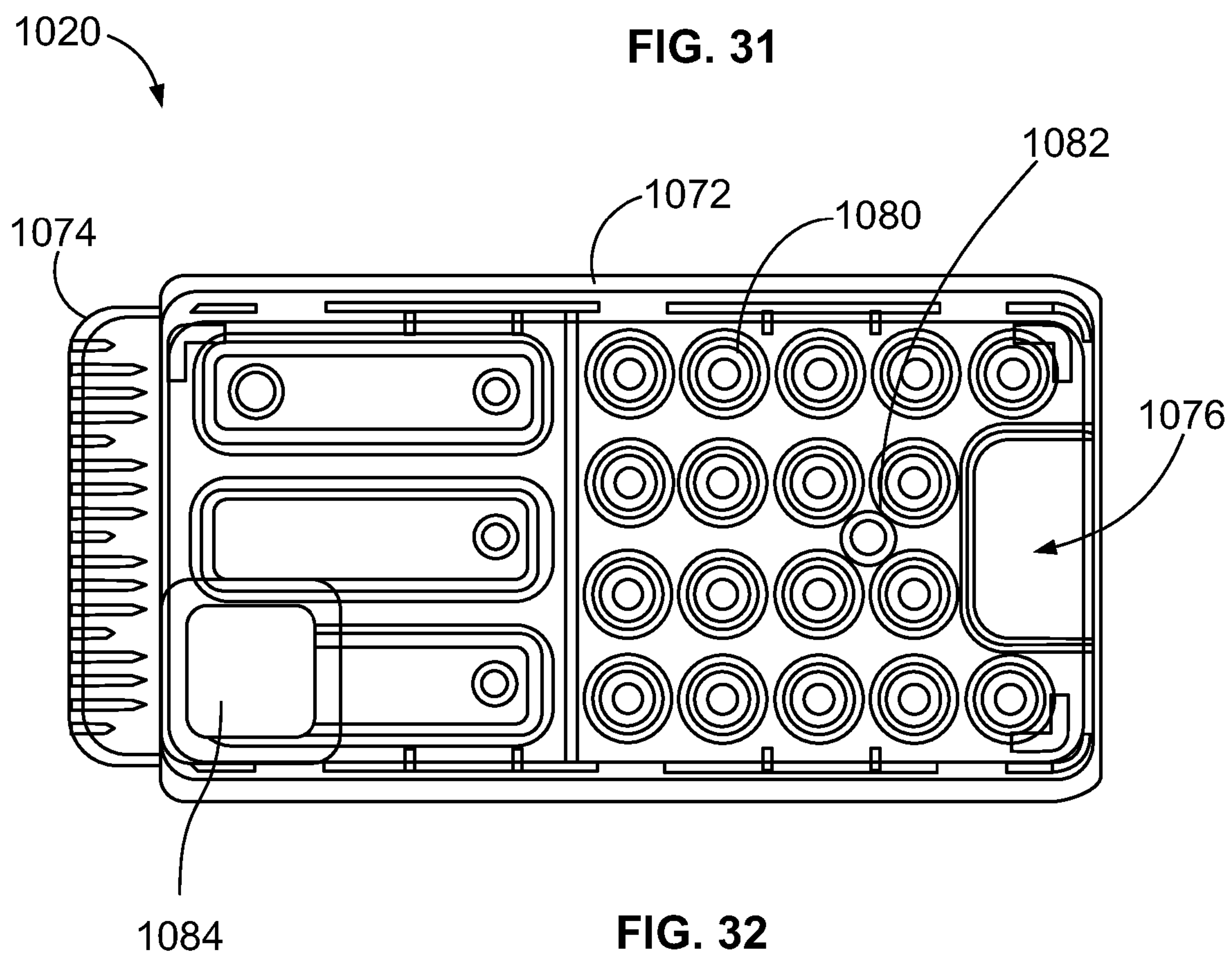


FIG. 32



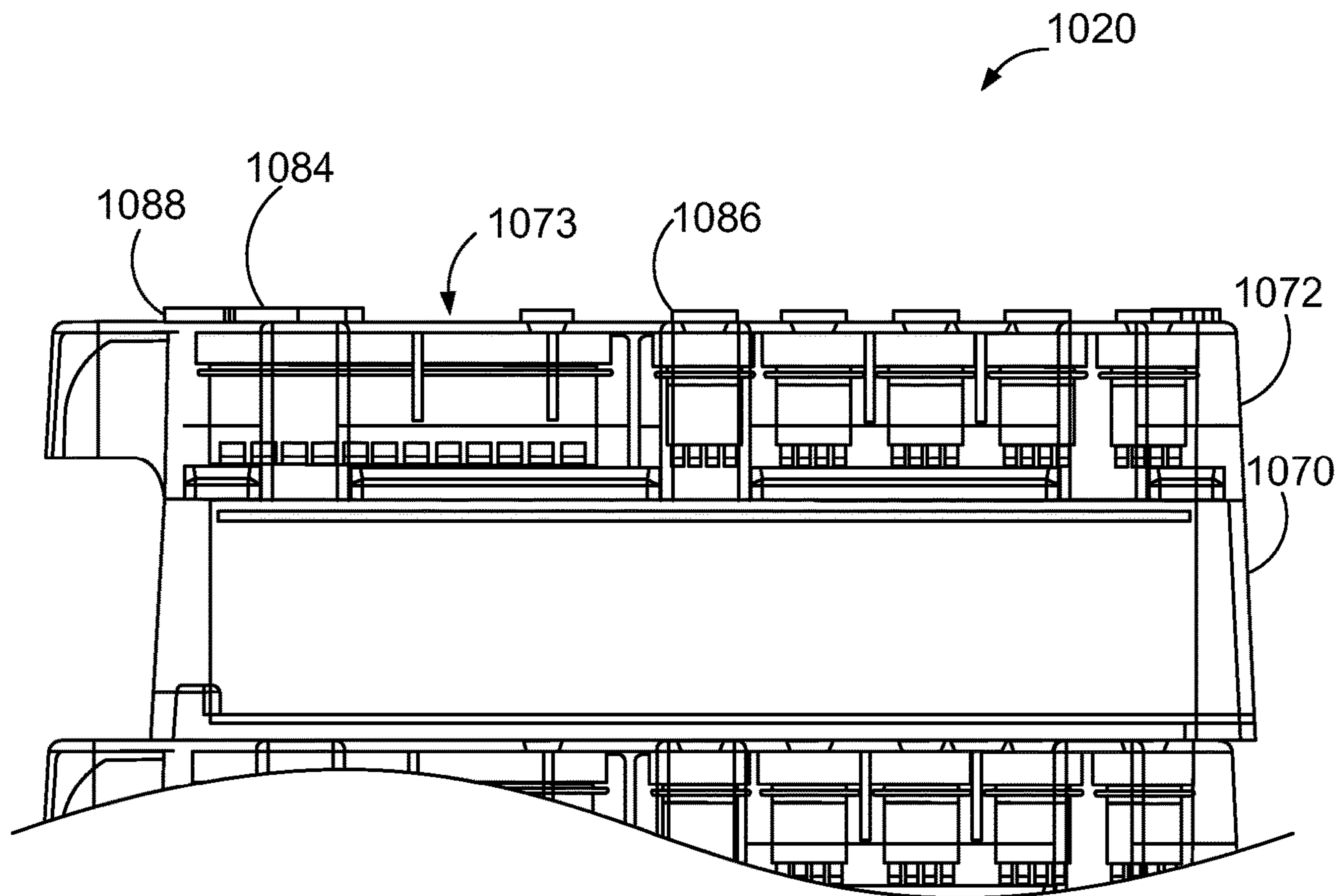


FIG. 33

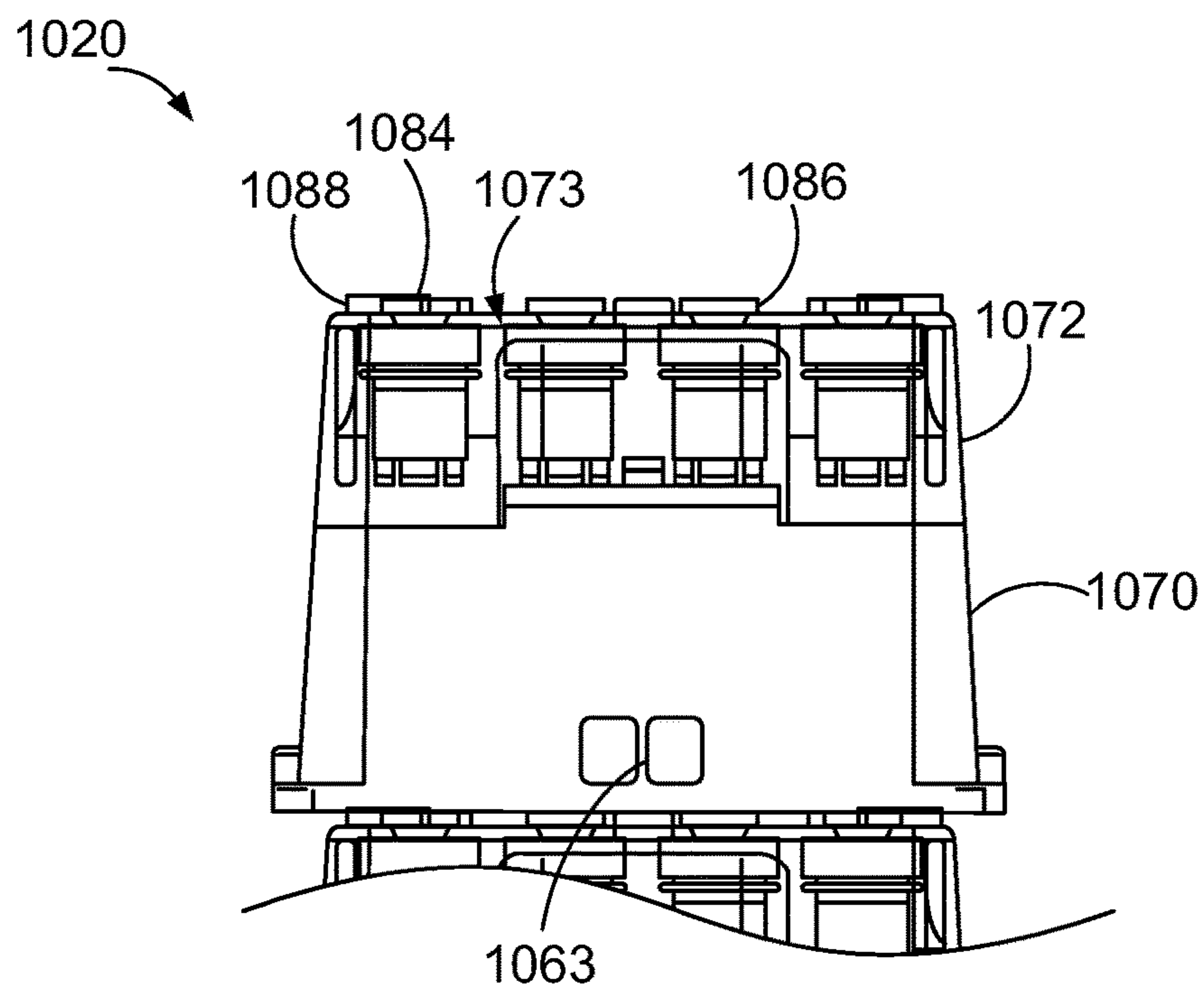


FIG. 34



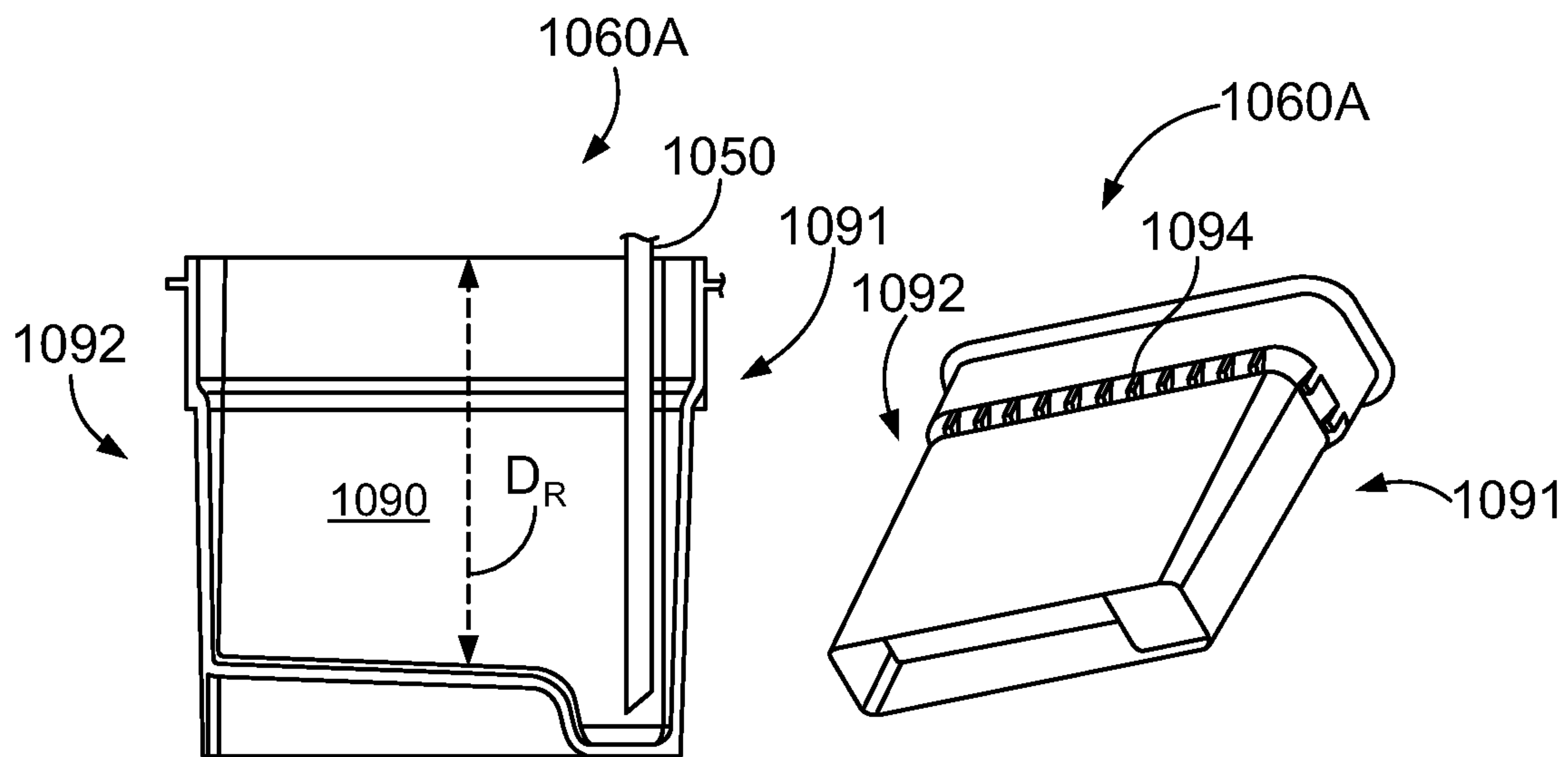


FIG. 36

FIG. 35

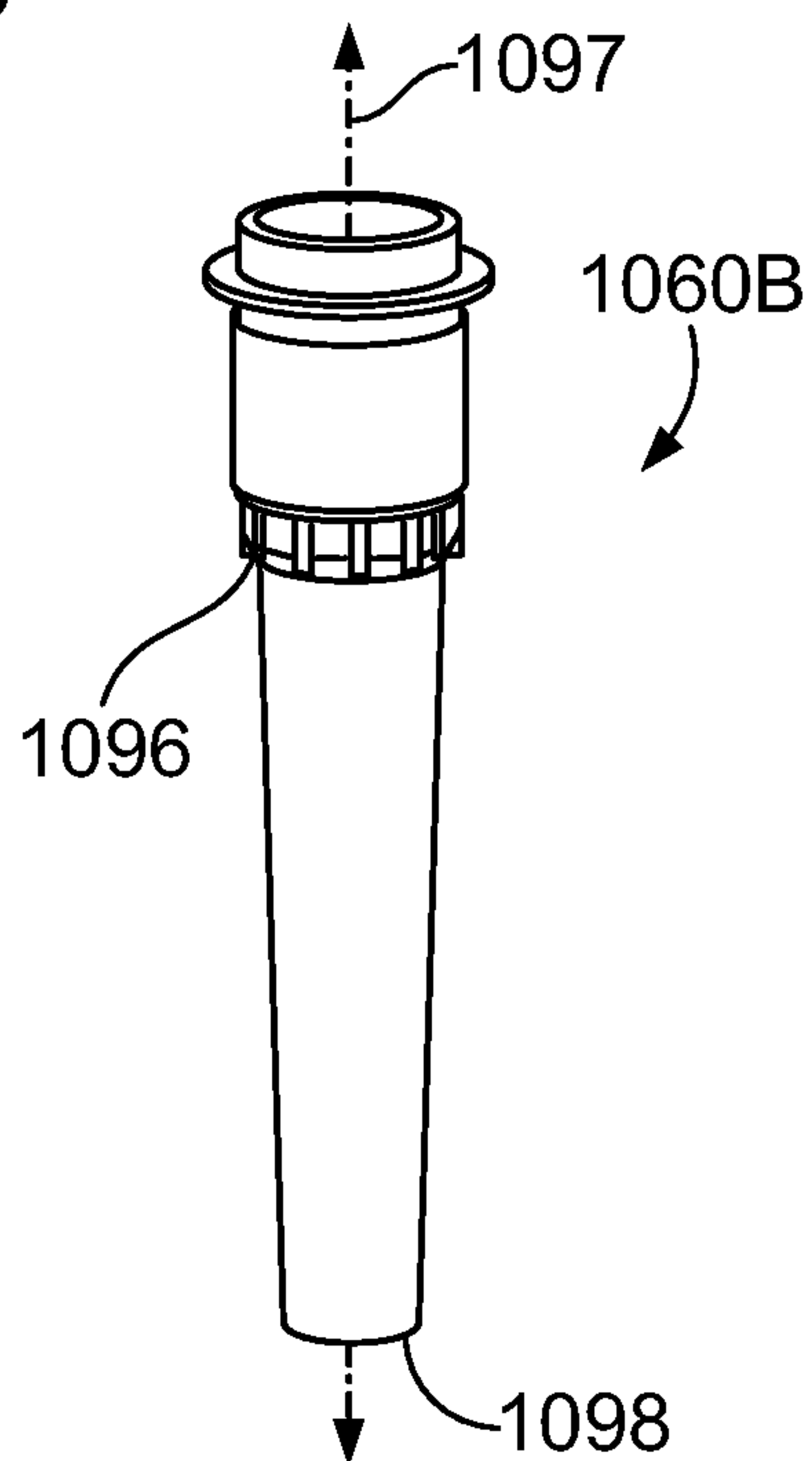


FIG. 37

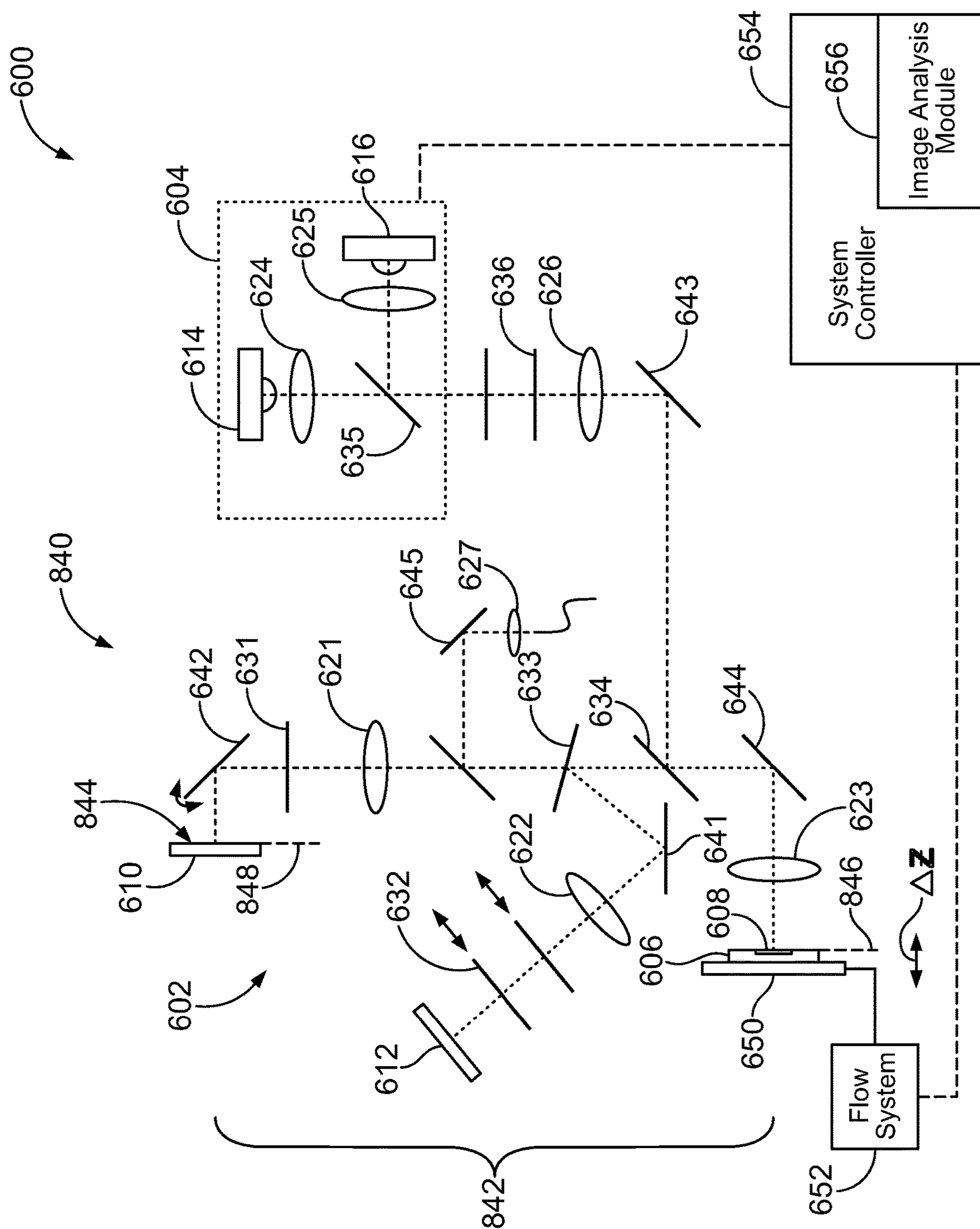


FIG. 38



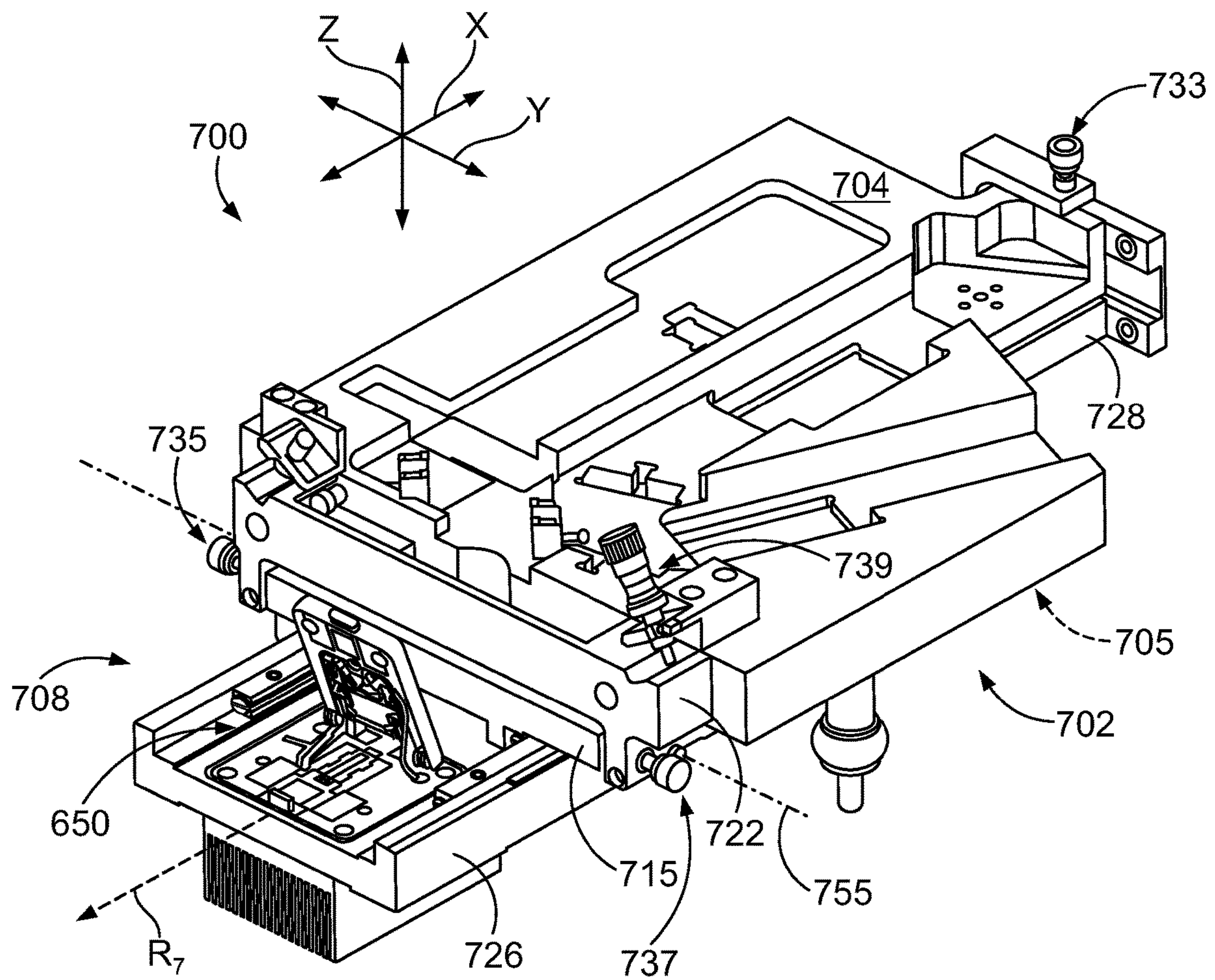


FIG. 39



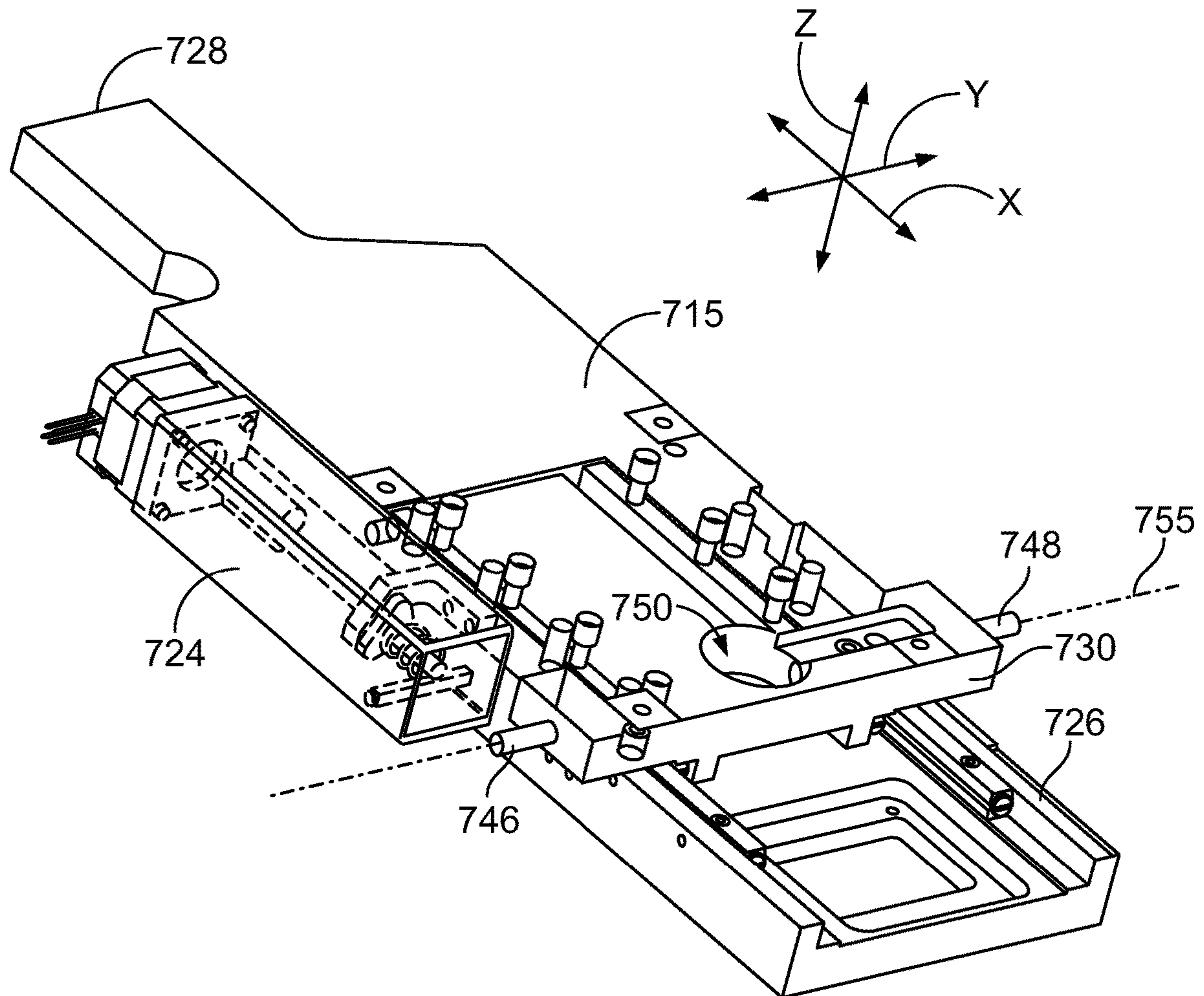


FIG. 40



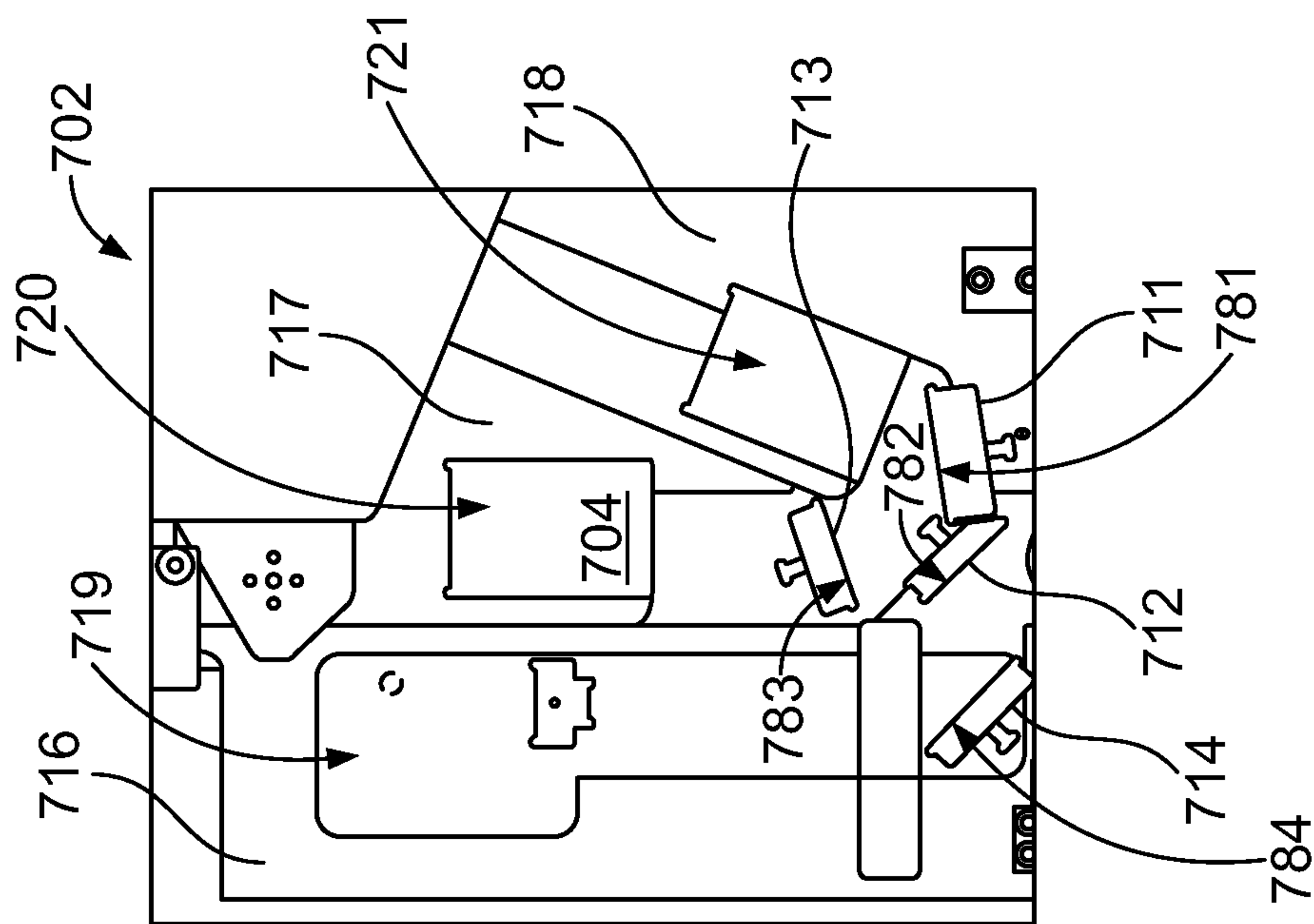


FIG. 42

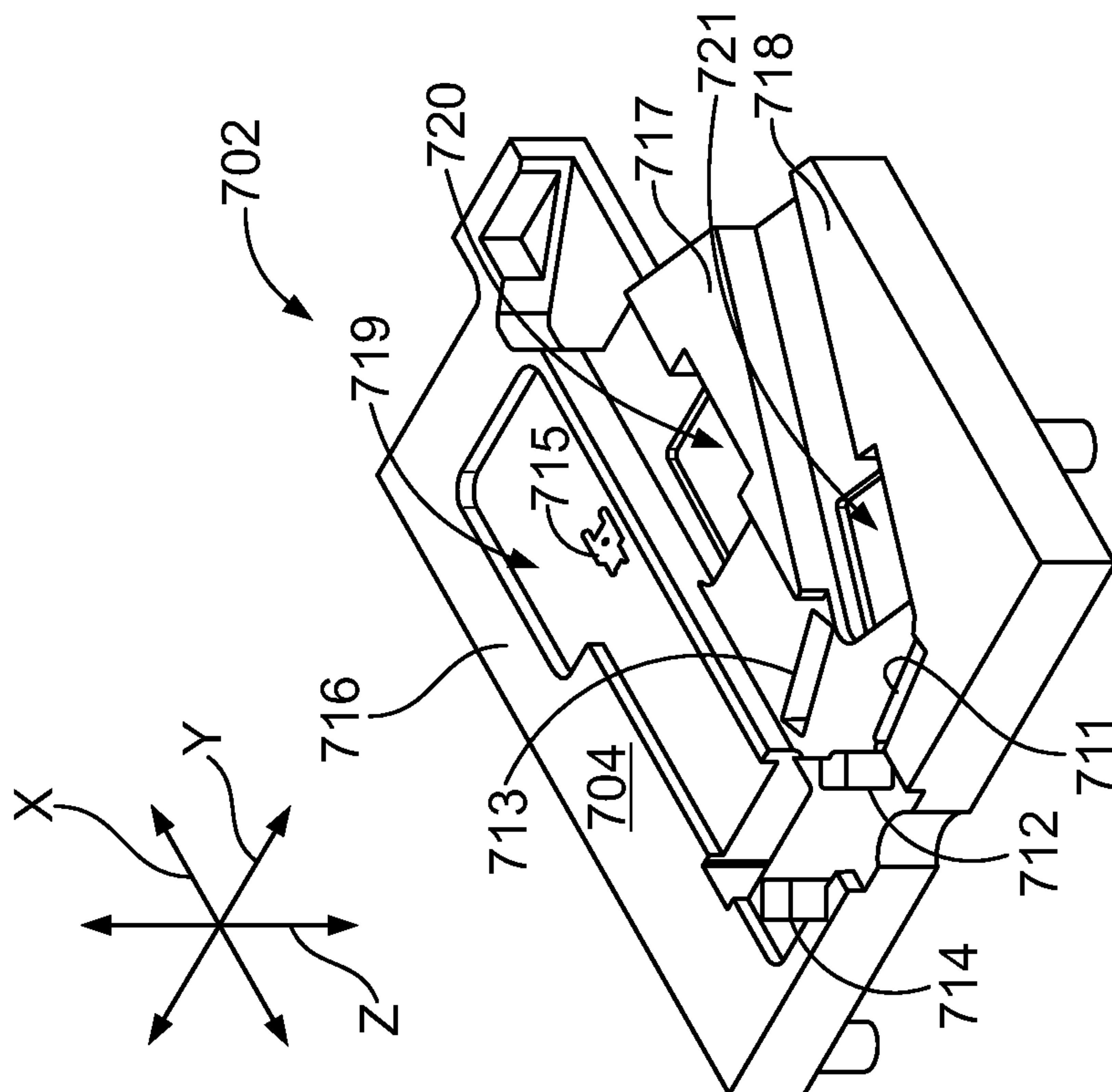


FIG. 41

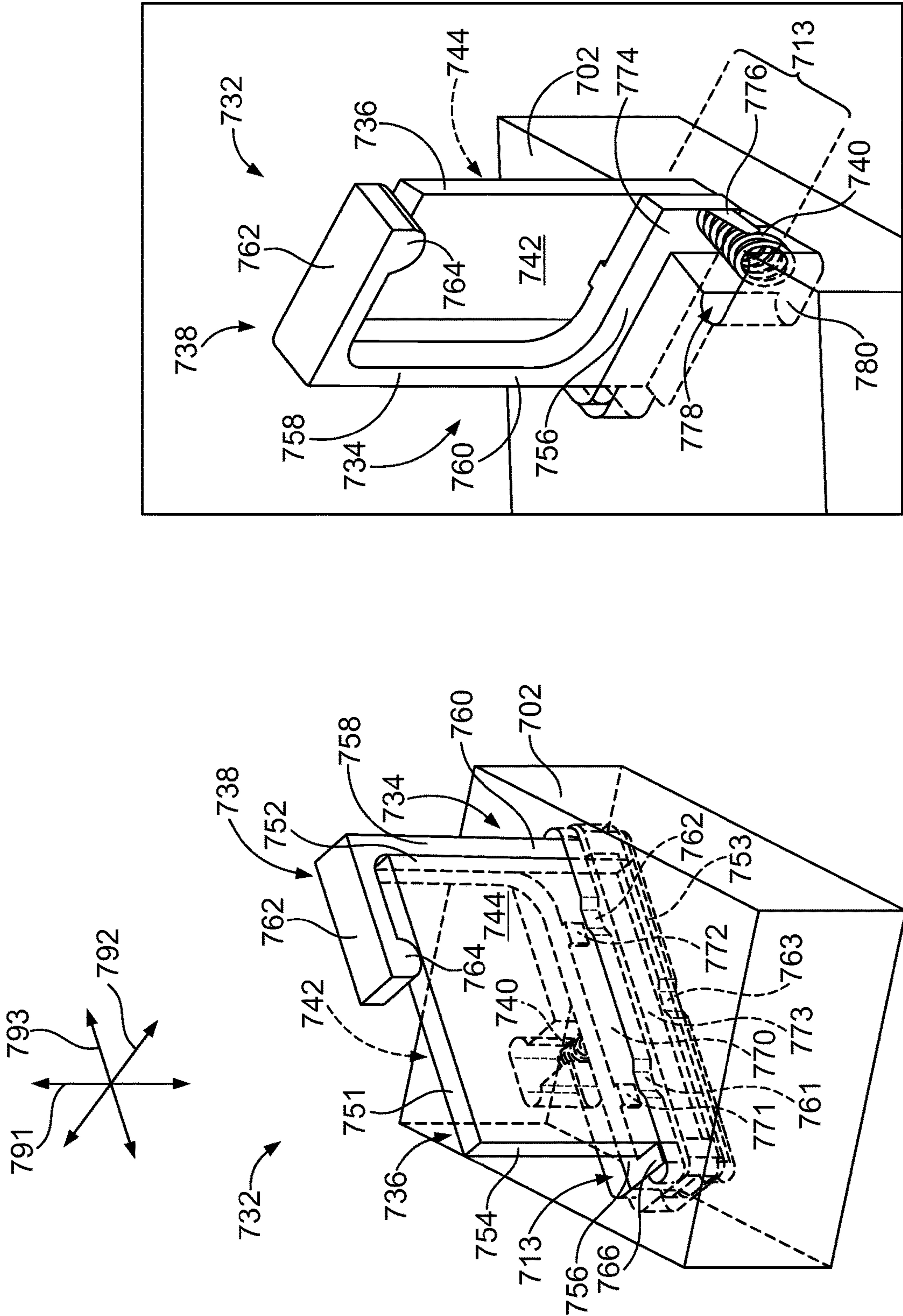


FIG. 44

FIG. 43



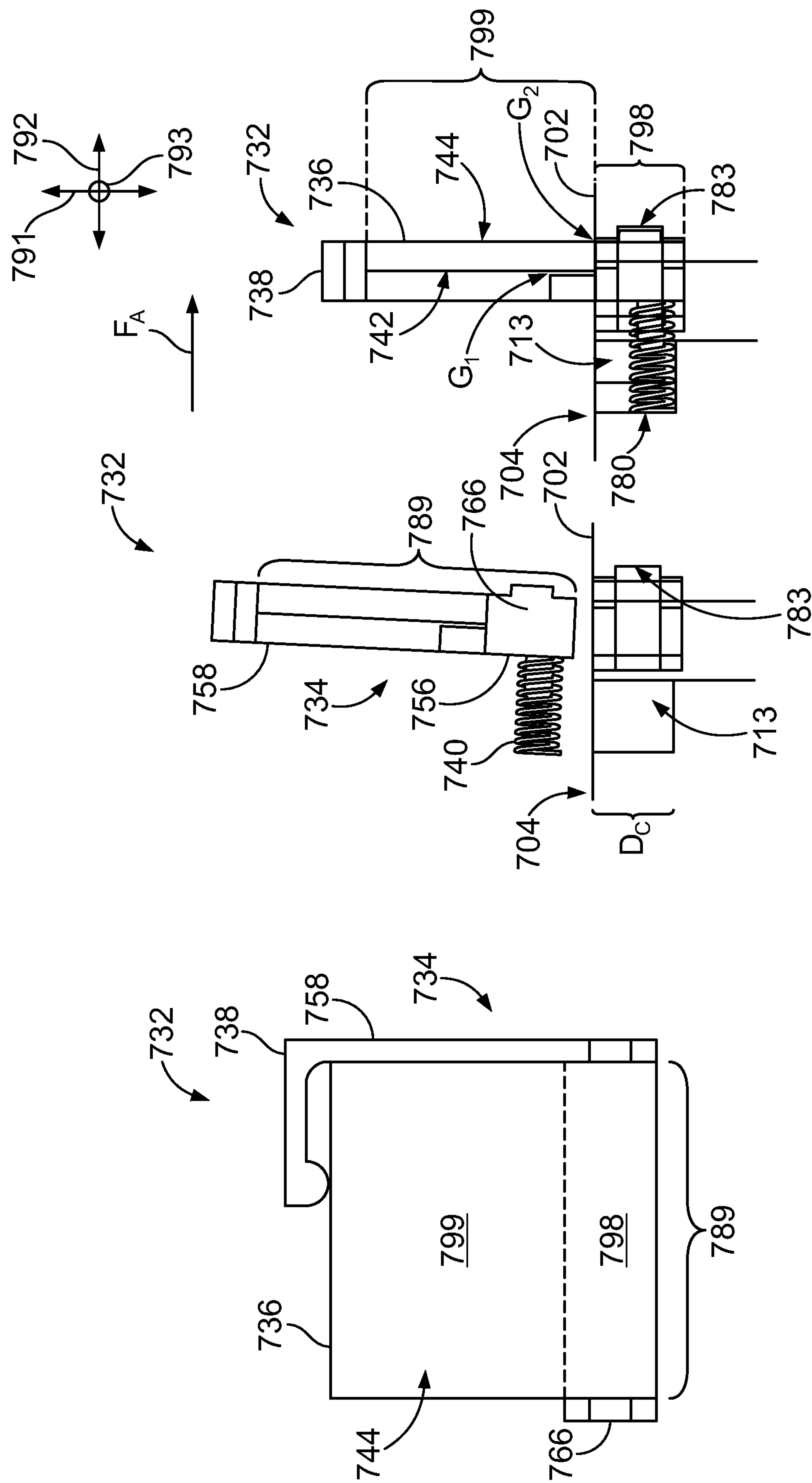
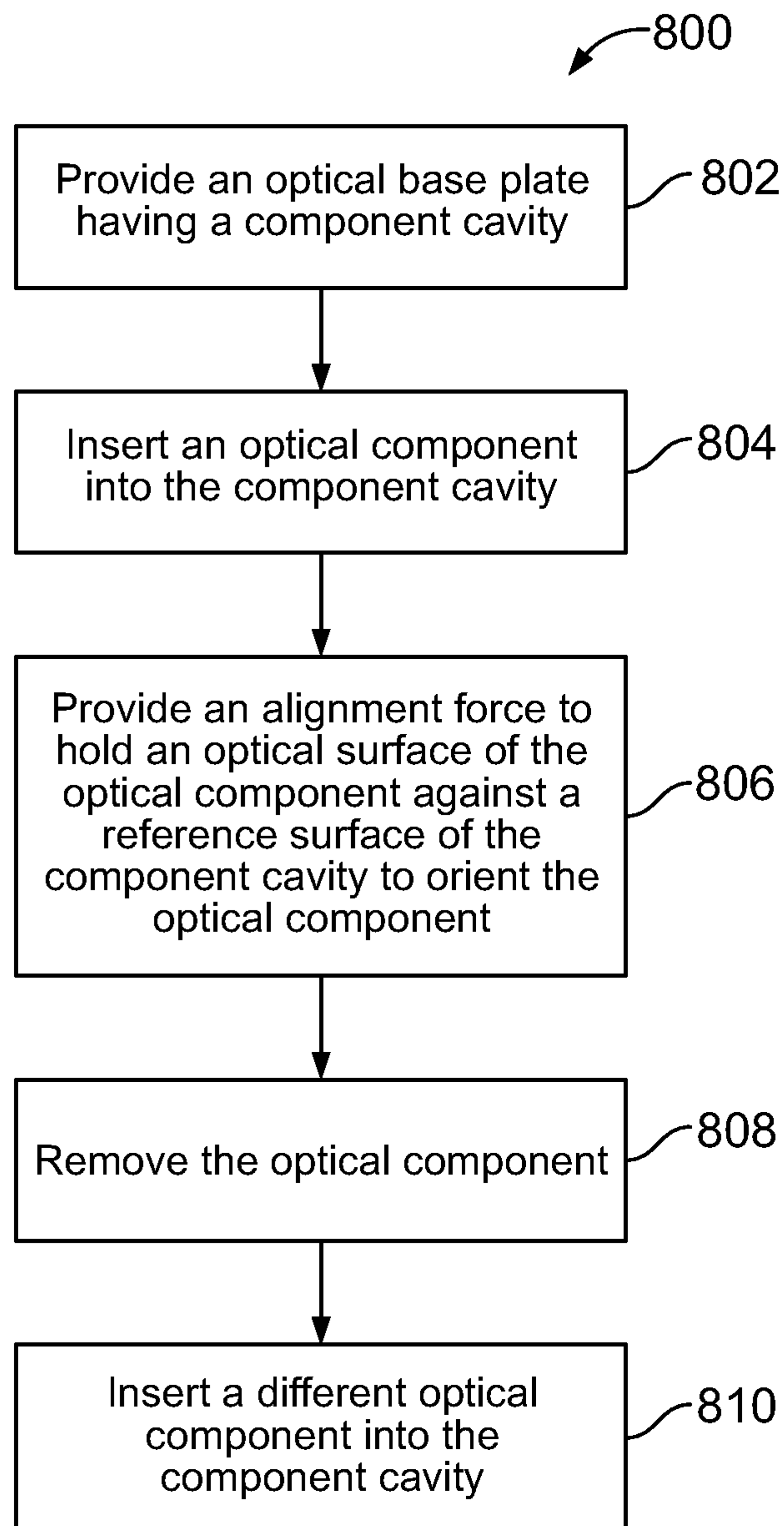


FIG. 46

FIG. 45

**FIG. 47**



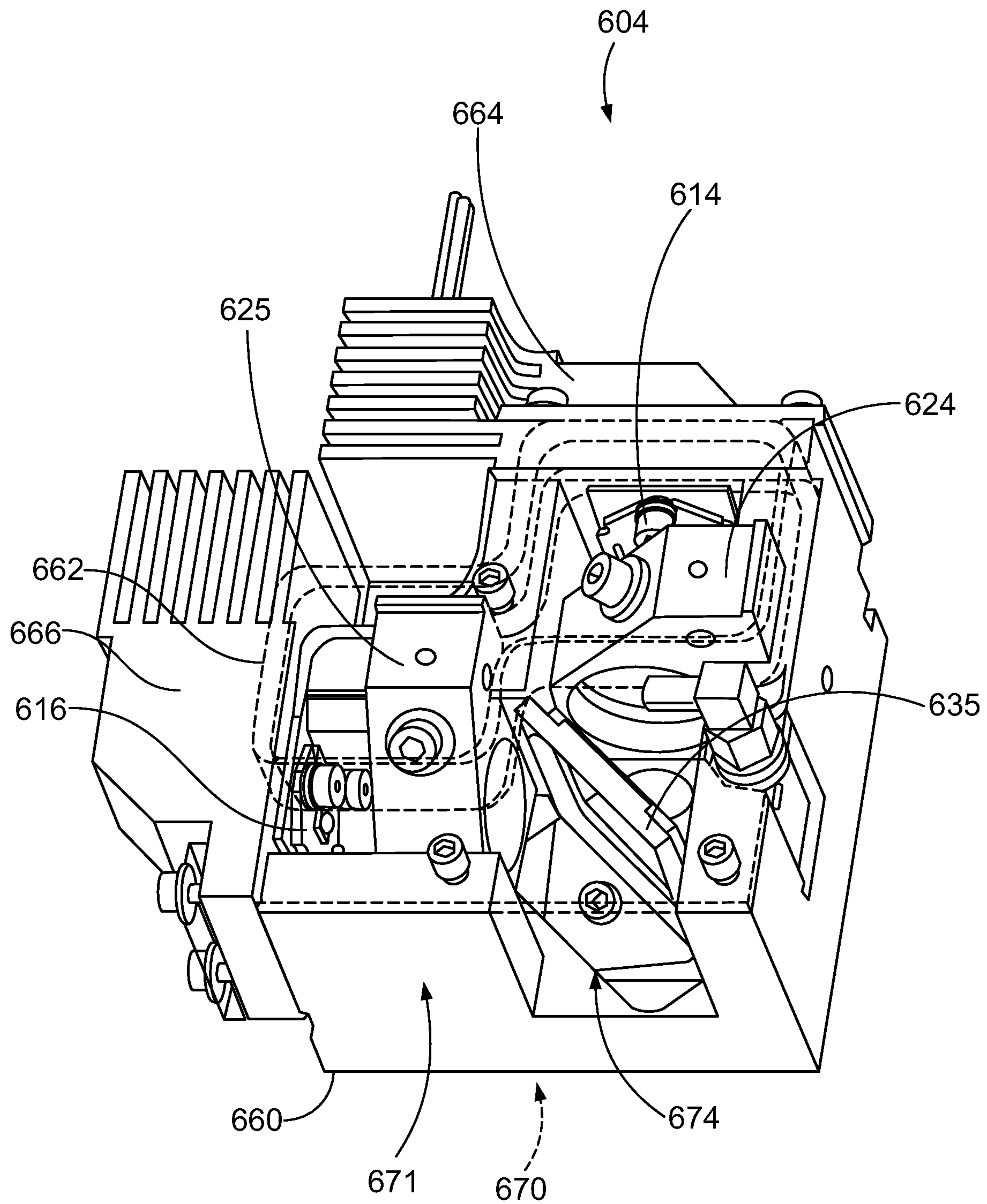


FIG. 48

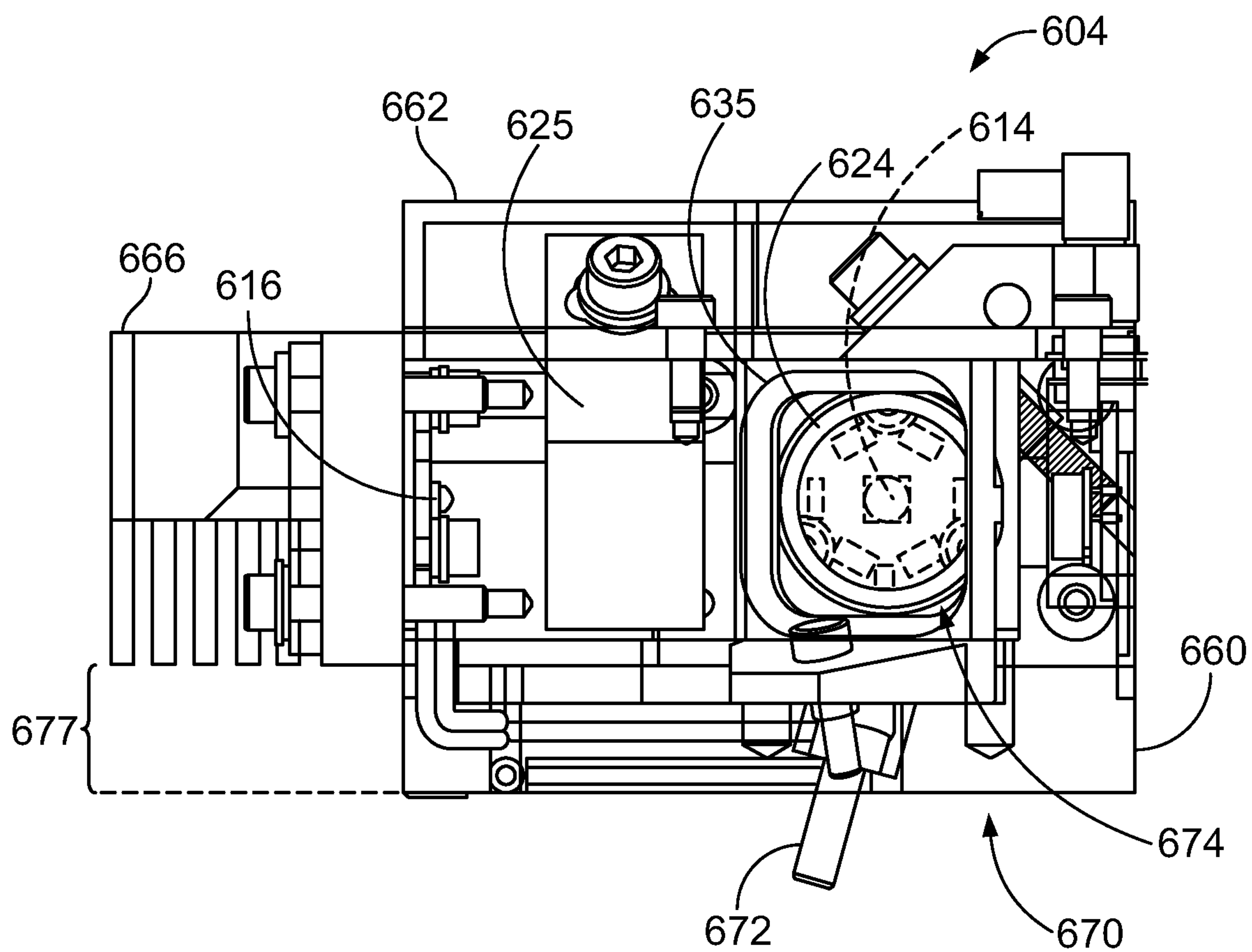


FIG. 49



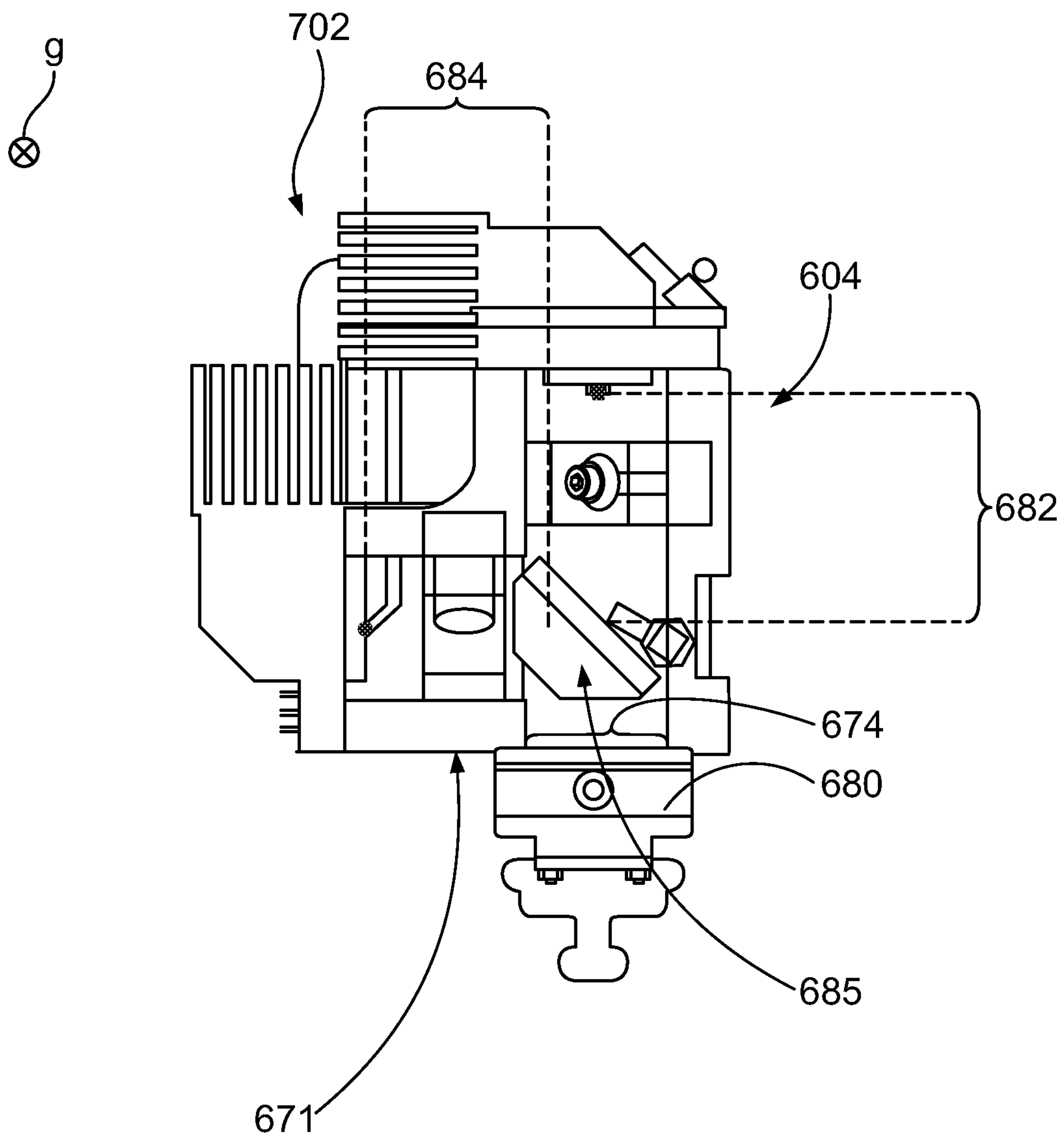


FIG. 50

**FIG. 51**



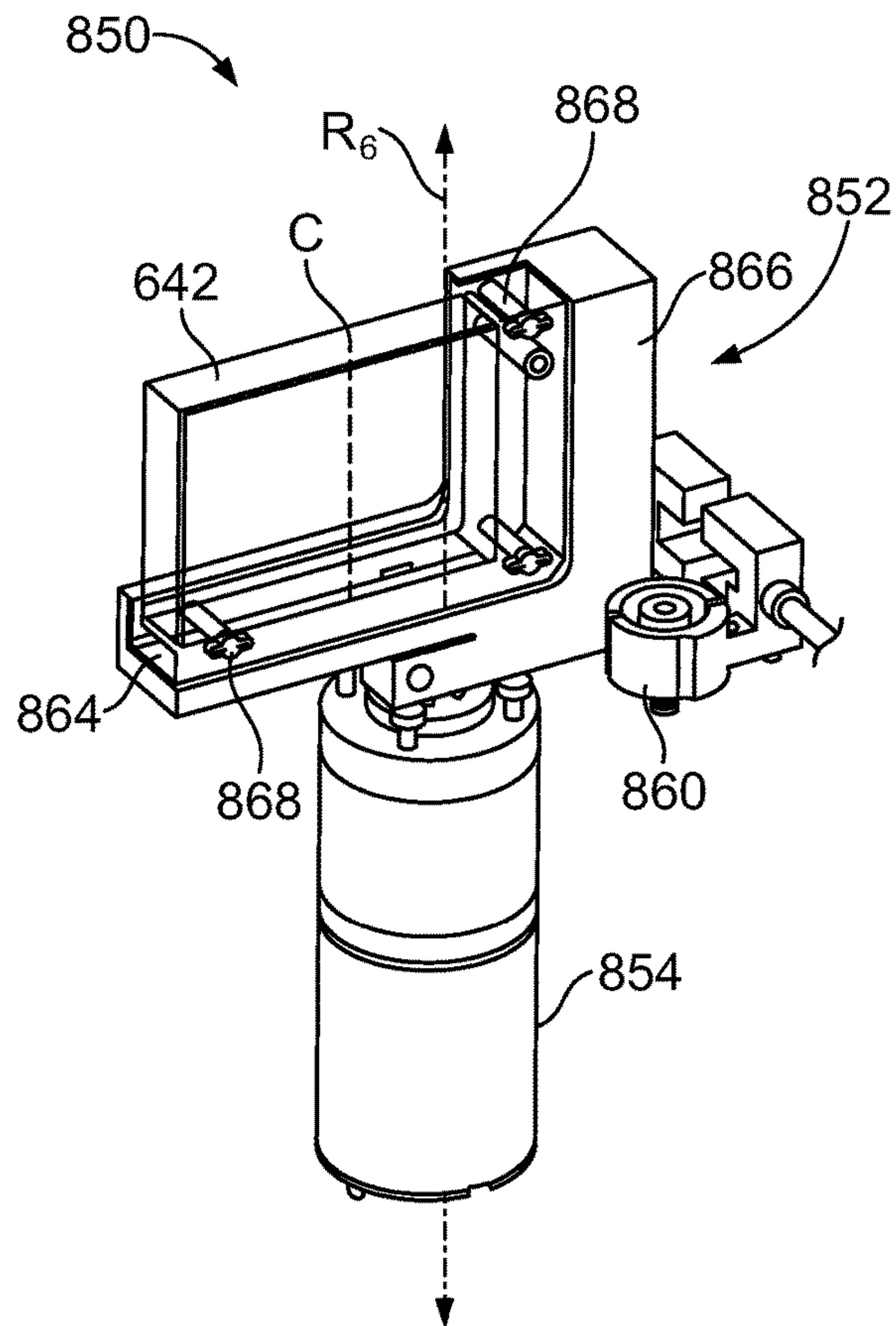


FIG. 52

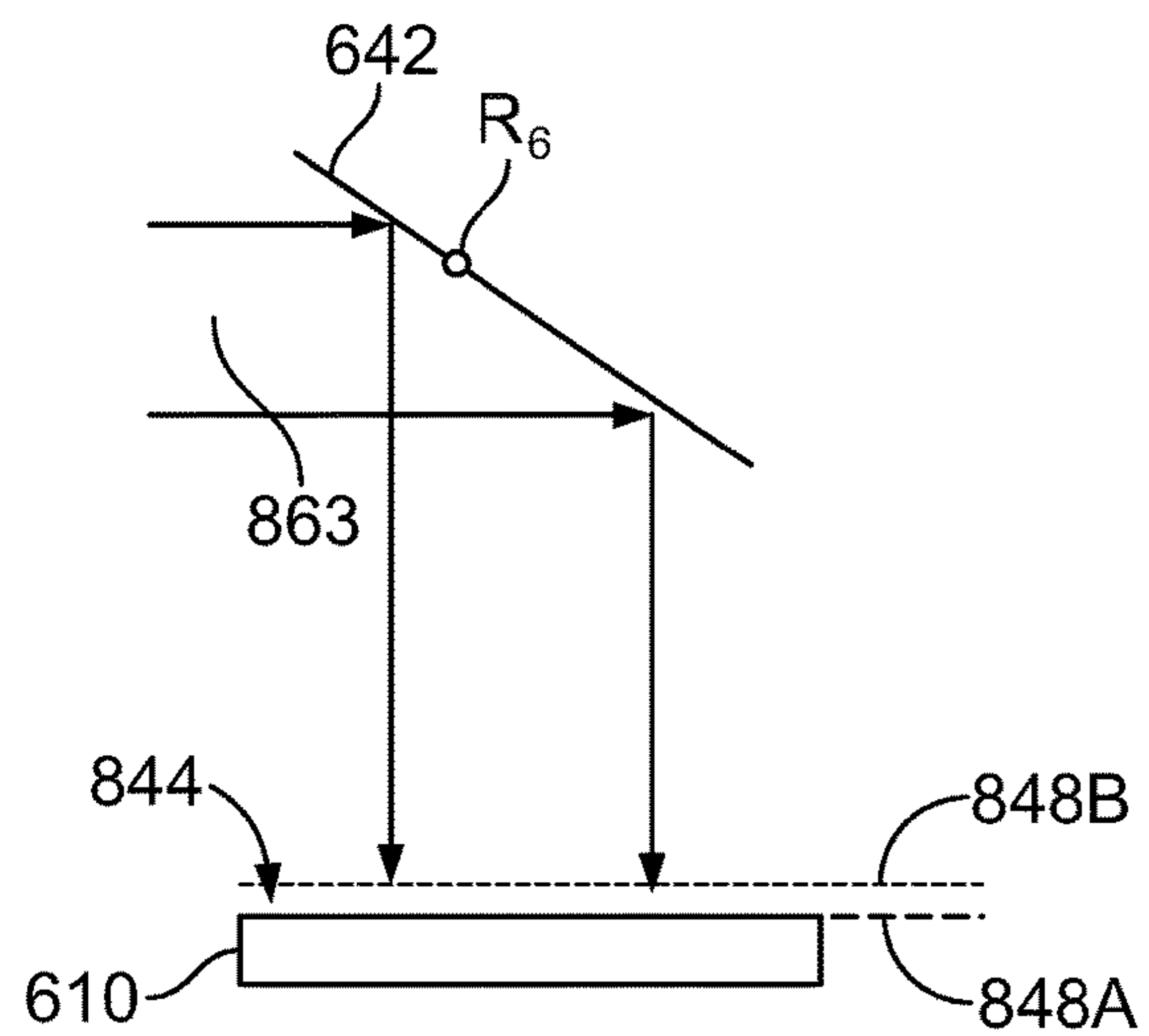


FIG. 53

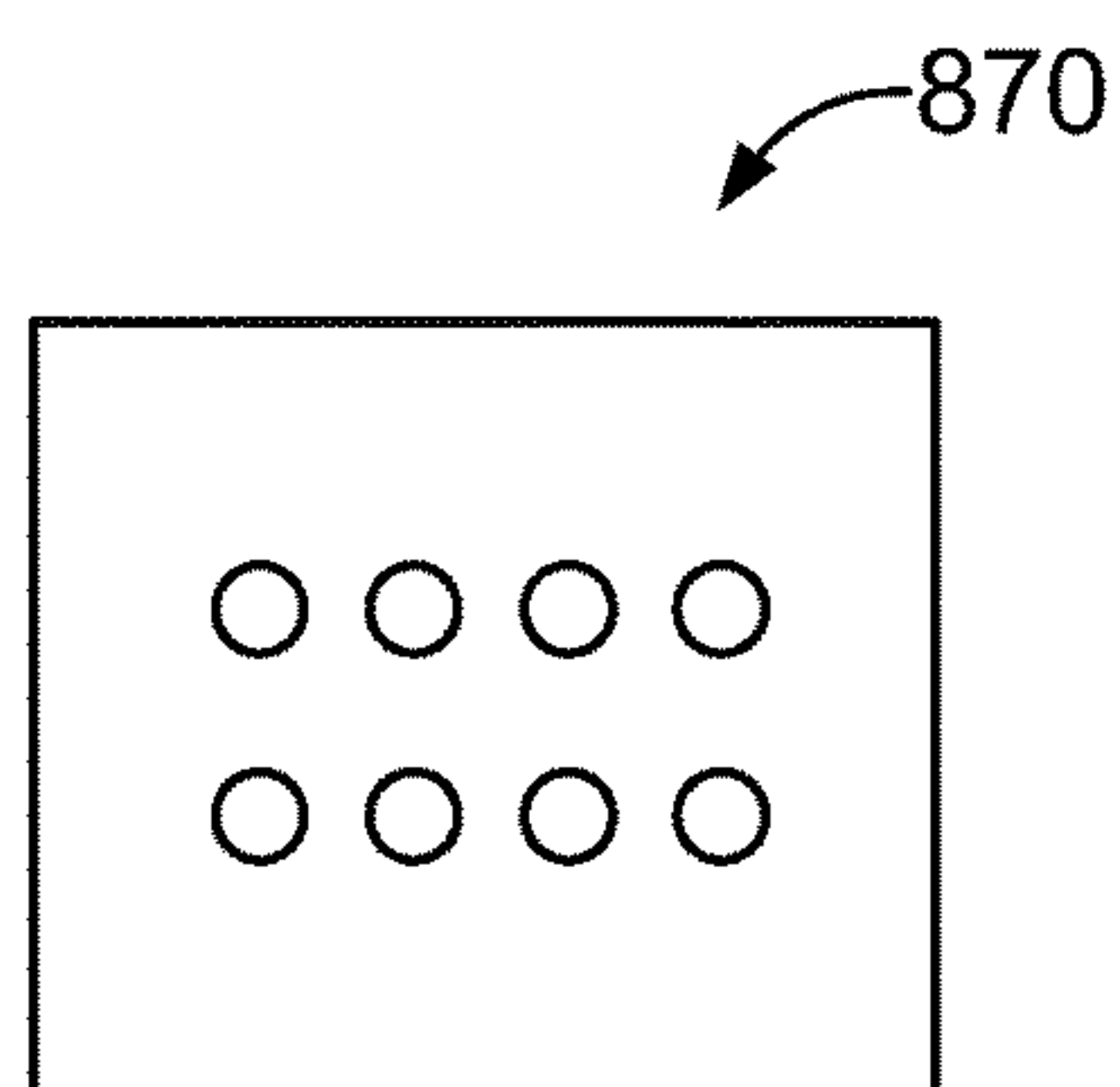


FIG. 54

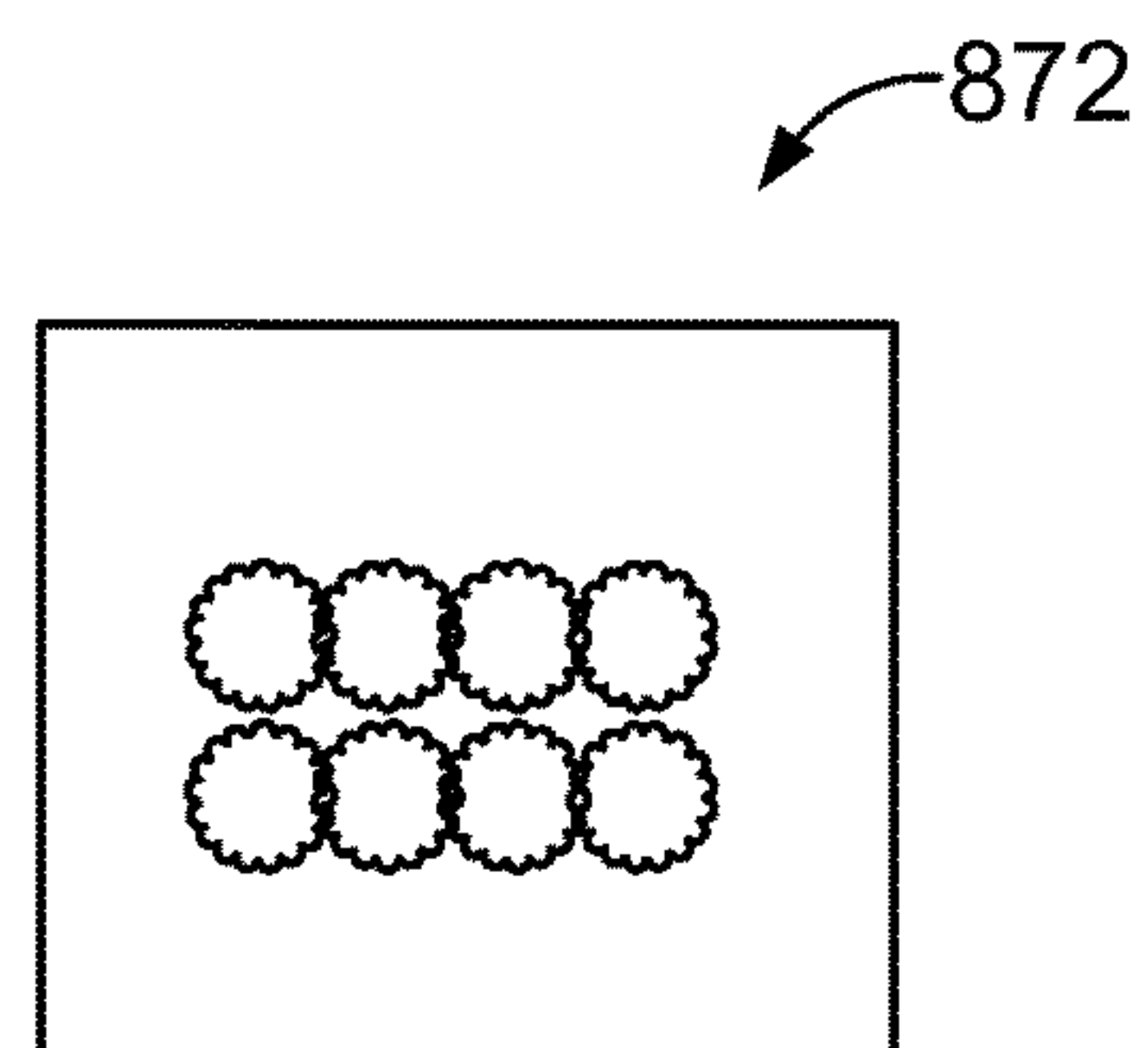


FIG. 55

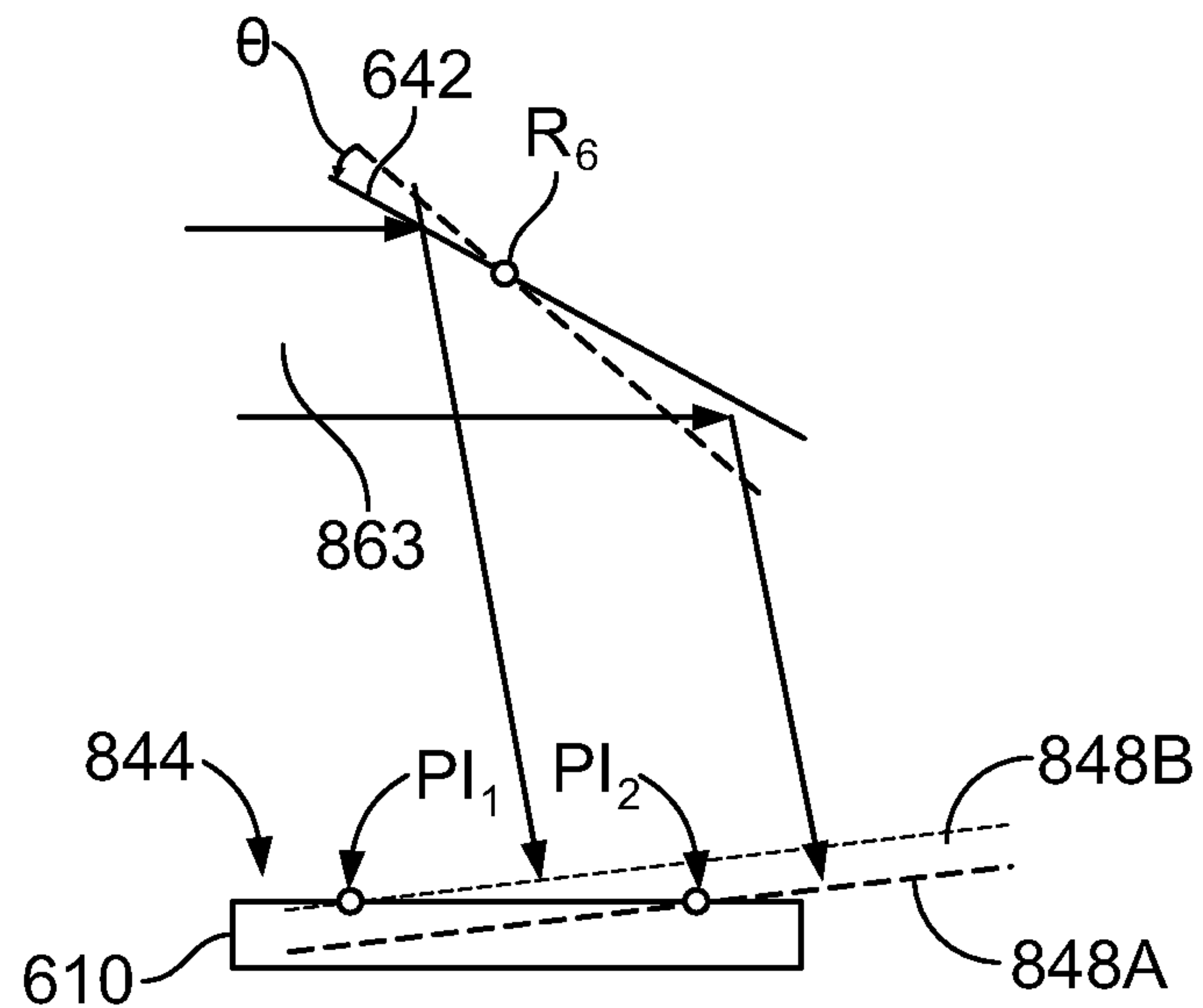


FIG. 56

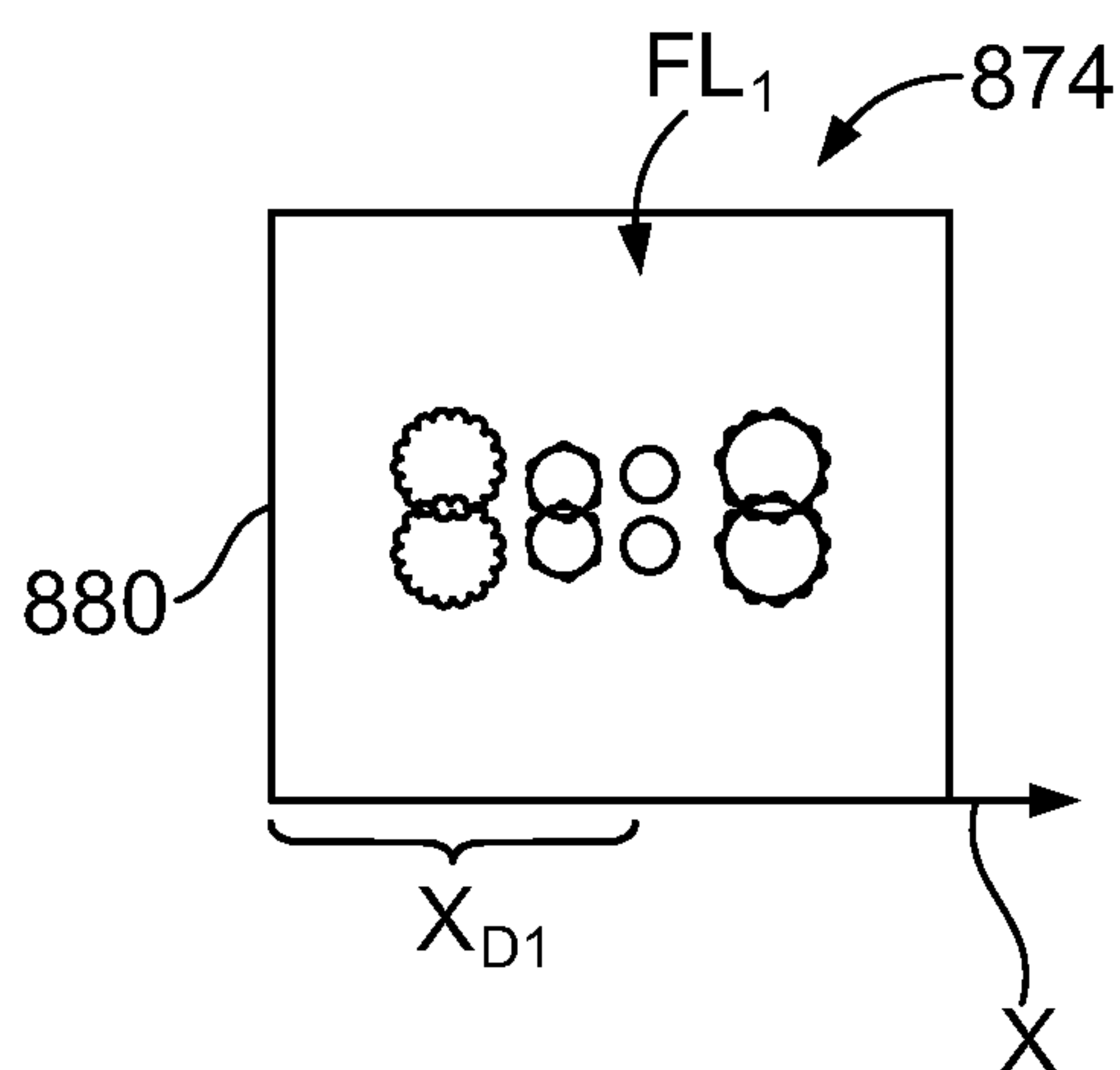


FIG. 57

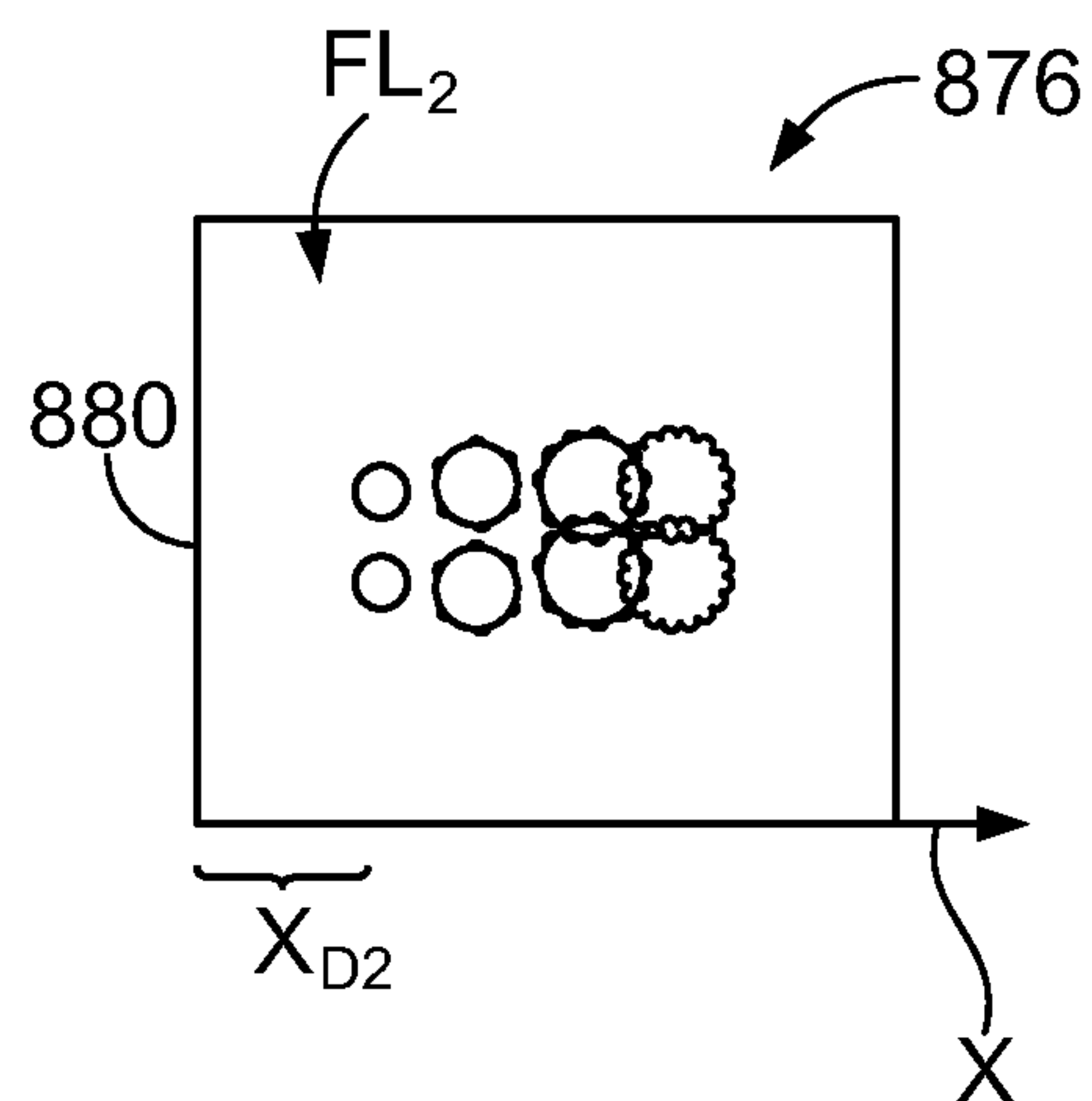


FIG. 58



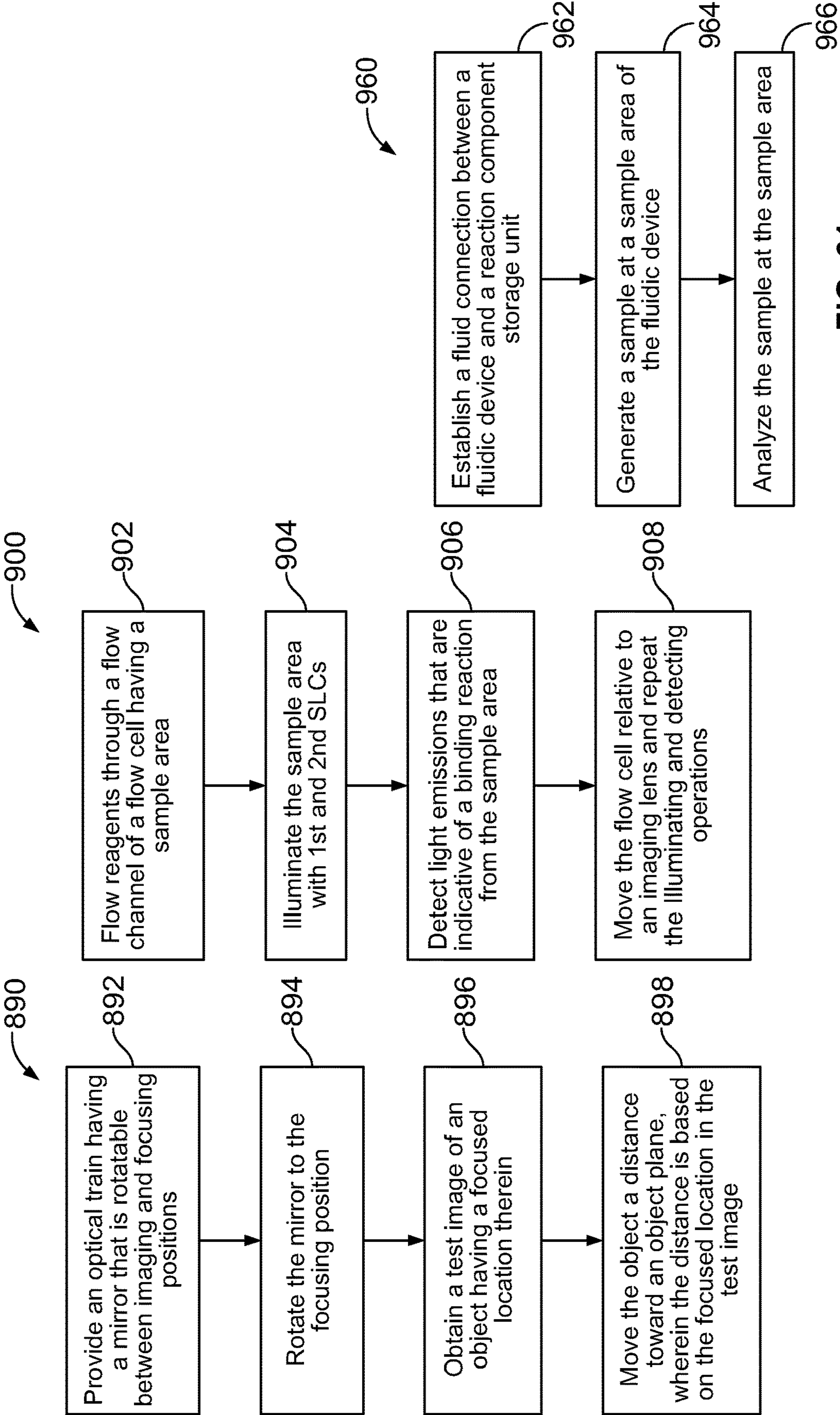


FIG. 59

FIG. 60

FIG. 61



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**SYSTEMS, METHODS, AND APPARATUSES  
TO IMAGE A SAMPLE FOR BIOLOGICAL  
OR CHEMICAL ANALYSIS****CROSS REFERENCE TO RELATED  
APPLICATIONS**

The present application is a continuation of U.S. patent application Ser. No. 18/144,485, filed May 8, 2023, which is a continuation of U.S. patent application Ser. No. 17/714, 129, filed Apr. 5, 2022, which is a continuation of U.S. patent application Ser. No. 16/255,546, filed Jan. 23, 2019, which is a divisional of U.S. application Ser. No. 14/550,956 (now U.S. Pat. No. 10,220,386), filed Nov. 22, 2014, which is a continuation of U.S. application Ser. No. 13/273,666 (Now U.S. Pat. No. 8,951,781), filed on Oct. 14, 2011, which relates to and claims the benefit of U.S. Provisional Application No. 61/431,425, filed on Jan. 10, 2011; U.S. Provisional Application No. 61/431,429, filed on Jan. 10, 2011; U.S. Provisional Application No. 61/431,439, filed on Jan. 11, 2011; U.S. Provisional Application No. 61/431,440, filed on Jan. 11, 2011; U.S. Provisional Application No. 61/438,486, filed on Feb. 1, 2011; U.S. Provisional Application No. 61/438,567, filed on Feb. 1, 2011; U.S. Provisional Application No. 61/438,530, filed on Feb. 1, 2011, the content of each of which is incorporated by reference herein in its entirety and for all purposes.

**BACKGROUND OF THE INVENTION**

Embodiments of the present invention relate generally to biological or chemical analysis and more particularly, to assay systems having fluidic devices, optical assemblies, and/or other apparatuses that may be used in detecting desired reactions in a sample.

Various assay protocols used for biological or chemical research are concerned with performing a large number of controlled reactions. In some cases, the controlled reactions are performed on support surfaces. The desired reactions may then be observed and analyzed to help identify properties or characteristics of the chemicals involved in the desired reaction. For example, in some protocols, a chemical moiety that includes an identifiable label (e.g., fluorescent label) may selectively bind to another chemical moiety under controlled conditions. These chemical reactions may be observed by exciting the labels with radiation and detecting light emissions from the labels. The light emissions may also be provided through other means, such as chemiluminescence.

Examples of such protocols include DNA sequencing. In one sequencing-by-synthesis (SBS) protocol, clusters of clonal amplicons are formed through bridge PCR on a surface of a flow channel. After generating the clusters of clonal amplicons, the amplicons may be “linearized” to make single stranded DNA (sstDNA). A series of reagents is flowed into the flow cell to complete a cycle of sequencing. Each sequencing cycle extends the sstDNA by a single nucleotide (e.g., A, T, G, C) having a unique fluorescent label. Each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in one cycle. After nucleotides are added to the sstDNAs clusters, an image in four channels is taken (i.e., one for each fluorescent label). After imaging, the fluorescent label and the terminator are chemically cleaved from the sstDNA and the growing DNA strand is ready for another cycle. Several cycles of reagent delivery and optical detection can be repeated to determine the sequences of the clonal amplicons.

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However, systems configured to perform such protocols may have limited capabilities and may not be cost-effective. Thus, there is a general need for improved systems, methods, and apparatuses that are capable of performing or being used during assay protocols, such as the SBS protocol described above, in a cost-effective, simpler, or otherwise improved manner.

**BRIEF DESCRIPTION OF THE INVENTION**

In accordance with one embodiment, a fluidic device for analyzing samples is provided. The fluidic device includes a flow cell having inlet and outlet ports and a flow channel extending therebetween. The flow cell is configured to hold a sample-of-interest. The fluidic device also includes a housing having a reception space that is configured to receive the flow cell. The reception space is sized and shaped to permit the flow cell to float relative to the housing. The fluidic device also includes a gasket that is coupled to the housing. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively.

In another embodiment, a removable cartridge configured to hold and facilitate positioning a flow cell for imaging is provided. The cartridge includes a removable housing that has a reception space configured to hold the flow cell substantially within an object plane. The housing includes a pair of housing sides that face in opposite directions. The reception space extends along at least one of the housing sides so that the flow cell is exposed to an exterior of the housing through said at least one of the housing sides. The cartridge also includes a cover member that is coupled to the housing and includes a gasket. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is configured to be mounted over an exposed portion of the flow cell when the flow cell is held by the housing.

In yet another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes positioning a removable fluidic device on a support surface of an imaging system. The device has a reception space, a flow cell located within the reception space, and a gasket. The flow cell extends along an object plane in the reception space and is floatable relative to the gasket within the object plane. The method also includes moving the flow cell within the reception space while on the support surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket.

In another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes providing a fluidic device having a housing that includes a reception space and a floatable flow cell located within the reception space. The housing has recesses that are located immediately adjacent to the reception space. The method also includes positioning the fluidic device on a support structure having alignment members. The alignment members are inserted through corresponding recesses. The method also includes moving the flow cell within the reception space. The alignment members engage edges of the flow cell when the flow cell is moved within the reception space.

In another embodiment, a fluidic device holder is provided that is configured to orient a sample area with respect to mutually perpendicular X, Y, and Z-axes. The device holder includes a support structure that is configured to receive a fluidic device. The support structure includes a



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base surface that faces in a direction along the Z-axis and is configured to have the device positioned thereon. The device holder also includes a plurality of reference surfaces in respective directions along an XY-plane and an alignment assembly that includes an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end toward and away from the reference surfaces. The locator arm is configured to hold the device against the reference surfaces when the locator arm is in the biased position.

In another embodiment, a fluidic device holder is provided that includes a support structure having a loading region for receiving a fluidic device. The support structure includes a base surface that partially defines the loading region and is configured to have the device positioned thereon. The device holder includes a cover assembly that is coupled to the support structure and is configured to be removably mounted over the device. The cover assembly includes a cover housing having housing legs and a bridge portion that joins the housing legs. The housing legs extend in a common direction and have a viewing space that is located therebetween. The viewing space is positioned above the loading region.

In another embodiment, a method for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes is provided. The method includes providing an alignment assembly that has a movable locator arm having an engagement end. The locator arm is movable between retracted and biased positions. The method also includes positioning a fluidic device on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. The device has a sample area. The method also includes moving the locator arm to the biased position. The locator arm presses the device against the reference surfaces such that the device is held in a fixed position.

In yet another embodiment, an optical assembly is provided that includes a base plate having a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The optical assembly also includes an optical component having an optical surface that is configured to reflect light or transmit light therethrough. The optical assembly also includes a mounting device that has a component retainer and a biasing element that is operatively coupled to the retainer. The retainer holds the optical component so that a space portion of the optical surface faces the reference surface and a path portion of the optical surface extends beyond the support side into an optical path. The biasing element provides an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, a method of assembling an optical train is provided. The method includes providing a base plate that has a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The method also includes inserting an optical component into the component-receiving space. The optical component has an optical surface that is configured to reflect light or transmit

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light therethrough. The optical surface has a space portion that faces the reference surface and a path portion that extends beyond the support side into an optical path. The method also includes providing an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, an optical imaging system is provided that includes an object holder to hold and move an object and a detector to detect optical signals from the object at a detector surface. The imaging system also includes an optical train that is configured to direct the optical signals onto the detector surface. The optical train has an object plane that is proximate to the object holder and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The imaging system also includes an image analysis module that is configured to analyze a test image detected at the detector surface when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location in the test image is indicative of a position of the object with respect to the object plane. The object holder is configured to move the object toward the object plane based on the focused location.

In another embodiment, a method for controlling focus of an optical imaging system is provided. The method includes providing an optical train that is configured to direct optical signals onto a detector surface. The optical train has an object plane that is proximate to an object and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The method also includes rotating the mirror to the focusing position and obtaining a test image of the object when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location is indicative of a position of the object with respect to the object plane. The method also includes moving the object toward the object plane based on the focused location.

In another embodiment, an optical imaging system is provided that includes a sample holder configured to hold a flow cell. The flow cell includes a flow channel having a sample area. The imaging system also includes a flow system that is coupled to the flow cell and configured to direct reagents through the flow channel to the sample area. The imaging system also includes an optical train that is configured to direct excitation light onto the sample area and first and second light sources. The first and second light sources have fixed positions with respect to the optical train. The first and second light sources provide first and second optical signals, respectively, for exciting the biomolecules. The imaging system also includes a system controller that is communicatively coupled to the first and second light sources and to the flow system. The controller is configured to activate the flow system to flow the reagents to the sample area and activate the first and second light sources after a predetermined synthesis time period. The light sources can be, for example, lasers or semiconductor light sources (SLSSs), such as laser diodes or light emitting diodes (LEDs).

In another embodiment, a method of performing a biological assay is provided. The method includes flowing reagents through a flow channel having a sample area. The



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sample area includes biomolecules that are configured to chemically react with the reagents. The method also includes illuminating the sample area with first and second light sources. The first and second light sources provide first and second optical signals, respectively. The biomolecules provide light emissions indicative of a binding reaction when illuminated by the first or second light sources. The method also includes detecting the light emissions from the sample area. The light sources can be, for example, lasers or semiconductor light sources (SLSs), such as a laser diodes or light emitting diodes (LEDs).

In another embodiment, a flow cell is provided that includes a first layer that has a mounting surface and an outer surface that face in opposite directions and that define a thickness therebetween. The flow cell also includes a second layer having a channel surface and an outer surface that face in opposite directions and that define a thickness therebetween. The second layer has a grooved portion that extends along the channel surface. The channel surface of the second layer is secured to the mounting surface. The flow cell also includes a flow channel that is defined by the grooved portion of the channel surface and a planar section of the mounting surface. The flow channel includes an imaging portion. The thickness of the second layer is substantially uniform along the imaging portion and is configured to transmit optical signals therethrough. The thickness of the first layer is substantially uniform along the imaging portion and is configured to permit uniform transfer of thermal energy therethrough.

In another embodiment, a light source module is provided that includes a module frame having a light passage and a light source that is secured to the module frame and oriented to direct optical signals through the light passage along an optical path. The light source module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the light source. The optical component is located within the light passage such that the optical component is within the optical path.

In another embodiment, an excitation light module is provided that includes a module frame and first and second semiconductor light sources (SLSs) that are secured to the module frame. The first and second SLSs have fixed positions with respect to each other. The first and second SLSs are configured to provide different excitation optical signals. The excitation light module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the first and second SLSs. The optical component permits the optical signals from the first SLS to transmit therethrough and reflects the optical signals from the second SLS. The reflected and transmitted optical signals are directed along a common path out of the module frame.

In one embodiment, a method of performing a biological or chemical assay is provided. The method includes establishing a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays. The reaction components include sample-generation components and sample-analysis components. The method also includes generating a sample at the sample area of the fluidic device. The generating operation includes flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. The method also includes analyzing the sample at the sample area. The analyzing operation includes flowing at least one sample-

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analysis component to the sample area. Said at least one sample-analysis component reacts with the sample to provide optically detectable signals indicative of an event-of-interest. The generating and analyzing operations are conducted in an automated manner by the assay system.

In another embodiment, an assay system is provided that includes a fluidic device holder that is configured to hold a fluidic device and establish a fluid connection with the fluidic device. The assay system also includes a fluidic network that is configured to fluidically connect the fluidic device to a reaction component storage unit. The assay system also includes a fluidic control system that is configured to selectively flow fluids from the storage unit through the fluidic device. Furthermore, the assay system includes a system controller that has a fluidic control module. The fluidic control module is configured to instruct the fluidic control system to (a) flow different sample-generation components from the storage unit to the sample area and control reaction conditions at the sample area to generate a sample; and (b) flow at least one sample-analysis component from the storage unit to the sample area. Said at least one sample-analysis component is configured to react with the sample to provide optically detectable signals indicative of an event-of-interest. The assay system also includes an imaging system that is configured to detect the optically detectable signals from the sample. The system controller is configured to automatically generate the sample and analyze the sample by selectively controlling the fluidic device holder, the fluidic control system, and the imaging system.

In another embodiment, a method of performing a biological or chemical assay is provided. The method includes: (a) providing a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays, the reaction components including sample-generation components and sample-analysis components; (b) flowing sample generation components according to a predetermined protocol to generate a sample at the sample area; (c) selectively controlling reaction conditions at the sample area to facilitate generating the sample; (d) flowing sample-analysis components to the sample area; and (e) detecting optical signals emitted from the sample area, the optical signals being indicative of an event-of-interest between the sample-analysis components and the sample; wherein (b)-(e) are conducted in an automated manner.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of an assay system for performing biological or chemical assays formed in accordance with one embodiment.

FIG. 2 is a side view of a workstation configured to perform biological or chemical assays in accordance with one embodiment.

FIG. 3 is a front view of the workstation of FIG. 2.

FIG. 4 is a diagram of a fluidic network formed in accordance with one embodiment.

FIG. 5 is a perspective view of a flow cell formed in accordance with one embodiment.

FIG. 6 is a cross-section of the flow cell shown in FIG. 5 taken along the line 6-6 in FIG. 5.

FIG. 7 is a plan view of the flow cell of FIG. 5.

FIG. 8 is an enlarged view of a curved segment of a flow channel.

FIG. 9 is a perspective view of a fluidic device formed in accordance with one embodiment.



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FIG. 10 is another perspective view of the fluidic device of FIG. 9.

FIG. 11 is a cross-section of the fluidic device of FIG. 9 taken along the lines 11-11 in FIG. 9.

FIG. 12 is a perspective view of a fluidic device formed in accordance with another embodiment.

FIG. 13 is a perspective view of the fluidic device of FIG. 12.

FIG. 14 is a plan view of a fluidic device formed in accordance with one embodiment.

FIG. 15 is a side perspective view of the fluidic device of FIG. 14.

FIG. 16 is a partially exploded view of a device holder formed in accordance with one embodiment.

FIG. 17 is a perspective view of the assembled holder of FIG. 16.

FIG. 18 is a perspective view of a support structure that may be used in the holder of FIG. 16.

FIG. 19 is a top plan view of the holder of FIG. 16.

FIG. 20 is a perspective view of the holder of FIG. 16 having a cover assembly in an open position.

FIG. 21 is an enlarged plan view of the holder of FIG. 16.

FIG. 22 is a perspective view of a cover assembly that may be used in the holder of FIG. 16.

FIG. 23 is a cross-section of the cover assembly taken along the line 23-23 shown in FIG. 22.

FIG. 24 is a perspective view of a flow system that may be used with the holder of FIG. 16.

FIG. 25 is a block diagram of a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 26 is a block diagram illustrating a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 27 is a block diagram illustrating a method for orienting a sample area in accordance with one embodiment.

FIG. 28 is a perspective view of a fluid storage system formed in accordance with one embodiment.

FIG. 29 is a side cross-section of the fluid storage system of FIG. 28.

FIG. 30 is a perspective view of a removal assembly that may be used with the fluid storage system of FIG. 28.

FIG. 31 is a perspective view of a reaction component tray formed in accordance with one embodiment.

FIG. 32 is a top plan view of the tray shown in FIG. 31.

FIG. 33 is a side view of the tray shown in FIG. 31.

FIG. 34 is a front view of the tray shown in FIG. 31.

FIG. 35 is a side cross-section of a component well that may be used with the tray of FIG. 31.

FIG. 36 is a bottom perspective view of the component well of FIG. 35.

FIG. 37 is a perspective view of a component well that may be used with the tray of FIG. 31.

FIG. 38 is a diagram of an optical imaging system in accordance with one embodiment.

FIG. 39 is a perspective view of a motion-control system in accordance with one embodiment.

FIG. 40 is a perspective view of components that may be used with the motion-control system of FIG. 39.

FIG. 41 is a perspective view of an optical base plate that may be used in the imaging system of FIG. 38.

FIG. 42 is a plan view of the base plate of FIG. 41.

FIG. 43 is a perspective view of an optical component formed in accordance with one embodiment that may be used in the imaging system of FIG. 38.

FIG. 44 is a cut-away perspective view of the optical component of FIG. 43.

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FIG. 45 is a front view of the optical component of FIG. 43.

FIG. 46 is a side view of the optical component of FIG. 43 during a mounting operation.

FIG. 47 is a block diagram illustrating a method of assembling an optical train in accordance with one embodiment.

FIG. 48 is a perspective view of a light source module formed in accordance with one embodiment.

FIG. 49 is a side view of the light source module of FIG. 48.

FIG. 50 is a plan view of the light source module of FIG. 48.

FIG. 51 is a plan view of an image-focusing system in accordance with one embodiment.

FIG. 52 is a perspective view of a rotatable mirror assembly that may be used in the image-focusing system of FIG. 51.

FIG. 53 is a schematic diagram of a rotatable mirror in an imaging position that may be used in the image-focusing system of FIG. 51.

FIGS. 54 and 55 illustrate sample images that may be obtained by the image-focusing system of FIG. 51.

FIG. 56 is a schematic diagram of the rotatable mirror of FIG. 53 in a focusing position.

FIGS. 57 and 58 illustrate test images that may be obtained by the image-focusing system of FIG. 51.

FIG. 59 is a block diagram illustrating a method for controlling focus of an optical imaging system.

FIG. 60 illustrates a method for performing an assay for biological or chemical analysis.

FIG. 61 illustrates a method for performing an assay for biological or chemical analysis.

#### DETAILED DESCRIPTION OF THE INVENTION

Embodiments described herein include various systems, methods, assemblies, and apparatuses used to detect desired reactions in a sample for biological or chemical analysis. In some embodiments, the desired reactions provide optical signals that are detected by an optical assembly. The optical signals may be light emissions from labels or may be transmission light that has been reflected or refracted by the sample. For example, embodiments may be used to perform or facilitate performing a sequencing protocol in which sstDNA is sequenced in a flow cell. In particular embodiments, the embodiments described herein can also perform an amplification protocol to generate a sample-of-interest for sequencing.

As used herein, a “desired reaction” includes a change in at least one of a chemical, electrical, physical, and optical property or quality of a substance that is in response to a stimulus. For example, the desired reaction may be a chemical transformation, chemical change, or chemical interaction. In particular embodiments, the desired reactions are detected by an imaging system. The imaging system may include an optical assembly that directs optical signals to a sensor (e.g., CCD or CMOS). However, in other embodiments, the imaging system may detect the optical signals directly. For example, a flow cell may be mounted onto a CMOS sensor. However, the desired reactions may also be a change in electrical properties. For example, the desired reaction may be a change in ion concentration within a solution.

Exemplary reactions include, but are not limited to, chemical reactions such as reduction, oxidation, addition,



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elimination, rearrangement, esterification, amidation, etherification, cyclization, or substitution; binding interactions in which a first chemical binds to a second chemical; dissociation reactions in which two or more chemicals detach from each other; fluorescence; luminescence; chemiluminescence; and biological reactions, such as nucleic acid replication, nucleic acid amplification, nucleic acid hybridization, nucleic acid ligation, phosphorylation, enzymatic catalysis, receptor binding, or ligand binding. The desired reaction can also be addition or elimination of a proton, for example, detectable as a change in pH of a surrounding solution or environment.

The stimulus can be at least one of physical, optical, electrical, magnetic, and chemical. For example, the stimulus may be an excitation light that excites fluorophores in a substance. The stimulus may also be a change in a surrounding environment, such as a change in concentration of certain biomolecules (e.g., enzymes or ions) in a solution. The stimulus may also be an electrical current applied to a solution within a predefined volume. In addition, the stimulus may be provided by shaking, vibrating, or moving a reaction chamber where the substance is located to create a force (e.g., centripetal force). As used herein, the phrase “in response to a stimulus” is intended to be interpreted broadly and include more direct responses to a stimulus (e.g., when a fluorophore emits energy of a specific wavelength after absorbing incident excitation light) and more indirect responses to a stimulus in that the stimulus initiates a chain of events that eventually results in the response (e.g., incorporation of a base in pyrosequencing eventually resulting in chemiluminescence). The stimulus may be immediate (e.g., excitation light incident upon a fluorophore) or gradual (e.g., change in temperature of the surrounding environment).

As used herein, the phrase “activity that is indicative of a desired reaction” and variants thereof include any detectable event, property, quality, or characteristic that may be used to facilitate determining whether a desired reaction has occurred. The detected activity may be a light signal generated in fluorescence or chemiluminescence. The detected activity may also be a change in electrical properties of a solution within a predefined volume or along a predefined area. The detected activity may be a change in temperature.

Various embodiments include providing a reaction component to a sample. As used herein, a “reaction component” or “reactant” includes any substance that may be used to obtain a desired reaction. For example, reaction components include reagents, enzymes, samples, other biomolecules, and buffer solutions. The reaction components are typically delivered to a reaction site (e.g., area where sample is located) in a solution or immobilized within a reaction site. The reaction components may interact directly or indirectly with the substance of interest.

In particular embodiments, the desired reactions are detected optically through an optical assembly. The optical assembly may include an optical train of optical components that cooperate with one another to direct the optical signals to an imaging device (e.g., CCD, CMOS, or photomultiplier tubes). However, in alternative embodiments, the sample region may be positioned immediately adjacent to an activity detector that detects the desired reactions without the use of an optical train. The activity detector may be able to detect predetermined events, properties, qualities, or characteristics within a predefined volume or area. For example, an activity detector may be able to capture an image of the predefined volume or area. An activity detector may be able to detect an ion concentration within a predefined volume of a solution or along a predefined area. Exemplary activity

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detectors include charged-coupled devices (CCD's) (e.g., CCD cameras); photomultiplier tubes (PMT's); molecular characterization devices or detectors, such as those used with nanopores; microcircuit arrangements, such as those described in U.S. Pat. No. 7,595,883, which is incorporated herein by reference in the entirety; and CMOS-fabricated sensors having field effect transistors (FET's), including chemically sensitive field effect transistors (chemFET), ion-sensitive field effect transistors (ISFET), and/or metal oxide semiconductor field effect transistors (MOSFET).

As used herein, the term “optical components” includes various elements that affect the propagation of optical signals. For example, the optical components may at least one of redirect, filter, shape, magnify, or concentrate the optical signals. The optical signals that may be affected include the optical signals that are upstream from the sample and the optical signals that are downstream from the sample. In a fluorescence-detection system, upstream components include those that direct excitation radiation toward the sample and downstream components include those that direct emission radiation away from the sample. Optical components may be, for example, reflectors, dichroics, beam splitters, collimators, lenses, filters, wedges, prisms, mirrors, detectors, and the like. Optical components also include bandpass filters, optical wedges, and optical devices similar to those described herein.

As used herein, the term “optical signals” or “light signals” includes electromagnetic energy capable of being detected. The term includes light emissions from labeled biological or chemical substances and also includes transmitted light that is refracted or reflected by optical substrates. Optical or light signals, including excitation radiation that is incident upon the sample and light emissions that are provided by the sample, may have one or more spectral patterns. For example, more than one type of label may be excited in an imaging session. In such cases, the different types of labels may be excited by a common excitation light source or may be excited by different excitation light sources at different times or at the same time. Each type of label may emit optical signals having a spectral pattern that is different from the spectral pattern of other labels. For example, the spectral patterns may have different emission spectra. The light emissions may be filtered to separately detect the optical signals from other emission spectra.

As used herein, when the term “different” is used with respect to light emissions (including emission spectra or other emission characteristics), the term may be interpreted broadly to include the light emissions being distinguishable or differentiable. For example, the emission spectra of the light emissions may have wavelength ranges that at least partially overlap so long as at least a portion of one emission spectrum does not completely overlap the other emission spectrum. Different emission spectra may also have the same or similar wavelength ranges, but have different intensities that are differentiable. Different optical signals can be distinguished based on different characteristics of excitation light that produces the optical signals. For example, in fluorescence resonance energy transfer (FRET) imaging, the light emissions may be the same but the cause (e.g., excitation optical signals) of the light emissions may be different. More specifically, a first excitation wavelength can be used to excite a donor fluorophore of a donor-acceptor pair such that FRET results in emission from the acceptor and excitation of the acceptor directly will also result in emission from the acceptor. As such, differentiation of the optical signals can be based on observation of an emission signal in combination with identification of the excitation wavelength



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used to produce the emission. Different light emissions may have other characteristics that do not overlap, such as emission anisotropy or fluorescence lifetime. Also, when the light emissions are filtered, the wavelength ranges of the emission spectra may be narrowed.

The optical components may have fixed positions in the optical assembly or may be selectively moveable. As used herein, when the term “selectively” is used in conjunction with “moving” and similar terms, the phrase means that the position of the optical component may be changed in a desired manner. At least one of the locations and the orientation of the optical component may be changed. For example, in particular embodiments, a rotatable mirror is selectively moved to facilitate focusing an optical imaging system.

Different elements and components described herein may be removably coupled. As used herein, when two or more elements or components are “removably coupled” (or “removably mounted,” and other like terms) the elements are readily separable without destroying the coupled components. For instance, elements can be readily separable when the elements may be separated from each other without undue effort, without the use of a tool (i.e. by hand), or without a significant amount of time spent in separating the components. By way of example, in some embodiments, an optical device may be removably mounted to an optical base plate. In addition, flow cells and fluidic devices may be removably mounted to a device holder.

Imaging sessions include a time period in which at least a portion of the sample is imaged. One sample may undergo or be subject to multiple imaging sessions. For example, one sample may be subject to two different imaging sessions in which each imaging session attempts to detect optical signals from one or more different labels. As a specific example, a first scan along at least a portion of a nucleic acid sample may detect labels associated with nucleotides A and C and a second scan along at least a portion of the sample may detect labels associated with nucleotides G and T. In sequencing embodiments, separate sessions can occur in separate cycles of a sequencing protocol. Each cycle can include one or more imaging session. In other embodiments, detecting optical signals in different imaging sessions may include scanning different samples. Different samples may be of the same type (e.g., two microarray chips) or of different types (e.g., a flow cell and a microarray chip).

During an imaging session, optical signals provided by the sample are observed. Various types of imaging may be used with embodiments described herein. For example, embodiments described herein may utilize a “step and shoot” procedure in which regions of a sample area are individually imaged. Embodiments may also be configured to perform at least one of epi-fluorescent imaging and total-internal-reflectance-fluorescence (TIRF) imaging. In other embodiments, the sample imager is a scanning time-delay integration (TDI) system. Furthermore, the imaging sessions may include “line scanning” one or more samples such that a linear focal region of light is scanned across the sample(s). Some methods of line scanning are described, for example, in U.S. Pat. No. 7,329,860 and U.S. Pat. Pub. No. 2009/0272914, each of which the complete subject matter is incorporated herein by reference in their entirety. Imaging sessions may also include moving a point focal region of light in a raster pattern across the sample(s). In alternative embodiments, imaging sessions may include detecting light emissions that are generated, without illumination, and based entirely on emission properties of a label within the sample (e.g., a radioactive or chemiluminescent component

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in the sample). In alternative embodiments, flow cells may be mounted onto an imager (e.g., CCD or CMOS) that detects the desired reactions.

As used herein, the term “sample” or “sample-of-interest” includes various materials or substances of interest that undergo an imaging session where optical signals from the material or substance are observed. In particular embodiments, a sample may include biological or chemical substances of interests and, optionally, an optical substrate or support structure that supports the biological or chemical substances. As such, a sample may or may not include an optical substrate or support structure. As used herein, the term “biological or chemical substances” may include a variety of biological or chemical substances that are suitable for being imaged or examined with the optical systems described herein. For example, biological or chemical substances include biomolecules, such as nucleosides, nucleic acids, polynucleotides, oligonucleotides, proteins, enzymes, polypeptides, antibodies, antigens, ligands, receptors, polysaccharides, carbohydrates, polyphosphates, nanopores, organelles, lipid layers, cells, tissues, organisms, and biologically active chemical compound(s) such as analogs or mimetics of the aforementioned species. Other chemical substances include labels that can be used for identification, examples of which include fluorescent labels and others set forth in further detail below.

Different types of samples may include different optical substrates or support structures that affect incident light in different manners. In particular embodiments, samples to be detected can be attached to one or more surfaces of a substrate or support structure. For example, flow cells may include one or more flow channels. In flow cells, the flow channels may be separated from the surrounding environment by top and bottom layers of the flow cell. Thus, optical signals to be detected are projected from within the support structure and may transmit through multiple layers of material having different refractive indices. For example, when detecting optical signals from an inner bottom surface of a flow channel and when detecting optical signals from above the flow channel, the optical signals that are desired to be detected may propagate through a fluid having an index of refraction, through one or more layers of the flow cells having different indices of refraction, and through the ambient environment having a different index of refraction.

As used herein, a “fluidic device” is an apparatus that includes one or more flow channels that direct fluid in a predetermined manner to conduct desired reactions. The fluidic device is configured to be fluidically coupled to a fluidic network of an assay system. By way of example, a fluidic device may include flow cells or lab-on-chip devices. Flow cells generally hold a sample along a surface for imaging by an external imaging system. Lab-on-chip devices may hold the sample and perform additional functions, such as detecting the desired reaction using an integrated detector. Fluidic devices may optionally include additional components, such as housings or imagers, that are operatively coupled to the flow channels. In particular embodiments, the channels may have channel surfaces where a sample is located, and the fluidic device can include a transparent material that permits the sample to be imaged after a desired reaction occurs.

In particular embodiments, the fluidic devices have channels with microfluidic dimensions. In such channels, the surface tension and cohesive forces of the liquid flowing therethrough and the adhesive forces between the liquid and the surfaces of the channel have at least a substantial effect on the flow of the liquid. For example, a cross-sectional area



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(taken perpendicular to a flow direction) of a microfluidic channel may be about  $10\ \mu\text{m}^2$  or less.

In alternative embodiments, optical imaging systems described herein may be used to scan samples that include microarrays. A microarray may include a population of different probe molecules that are attached to one or more substrates such that the different probe molecules can be differentiated from each other according to relative location. An array can include different probe molecules, or populations of the probe molecules, that are each located at a different addressable location on a substrate. Alternatively, a microarray can include separate optical substrates, such as beads, each bearing a different probe molecule, or population of the probe molecules, that can be identified according to the locations of the optical substrates on a surface to which the substrates are attached or according to the locations of the substrates in a liquid. Exemplary arrays in which separate substrates are located on a surface include, without limitation, a BeadChip Array available from Illumina®, Inc. (San Diego, CA) or others including beads in wells such as those described in U.S. Pat. Nos. 6,266,459, 6,355,431, 6,770,441, 6,859,570, and 7,622,294; and PCT Publication No. WO 00/63437, each of which is hereby incorporated by reference. Other arrays having particles on a surface include those set forth in US 2005/0227252; WO 05/033681; and WO 04/024328, each of which is hereby incorporated by reference.

Any of a variety of microarrays known in the art can be used. A typical microarray contains sites, sometimes referred to as features, each having a population of probes. The population of probes at each site is typically homogenous having a single species of probe, but in some embodiments the populations can each be heterogeneous. Sites or features of an array are typically discrete, being separated. The separate sites can be contiguous or they can have spaces between each other. The size of the probe sites and/or spacing between the sites can vary such that arrays can be high density, medium density or lower density. High density arrays are characterized as having sites separated by less than about  $15\ \mu\text{m}$ . Medium density arrays have sites separated by about  $15$  to  $30\ \mu\text{m}$ , while low density arrays have sites separated by greater than  $30\ \mu\text{m}$ . An array useful in the invention can have sites that are separated by less than  $100\ \mu\text{m}$ ,  $50\ \mu\text{m}$ ,  $10\ \mu\text{m}$ ,  $5\ \mu\text{m}$ ,  $1\ \mu\text{m}$ , or  $0.5\ \mu\text{m}$ . An apparatus or method of an embodiment of the invention can be used to image an array at a resolution sufficient to distinguish sites at the above densities or density ranges.

Further examples of commercially available microarrays that can be used include, for example, an Affymetrix® GeneChip® microarray or other microarray synthesized in accordance with techniques sometimes referred to as VLSIPS™ (Very Large Scale Immobilized Polymer Synthesis) technologies as described, for example, in U.S. Pat. Nos. 5,324,633; 5,744,305; 5,451,683; 5,482,867; 5,491,074; 5,624,711; 5,795,716; 5,831,070; 5,856,101; 5,858,659; 5,874,219; 5,968,740; 5,974,164; 5,981,185; 5,981,956; 6,025,601; 6,033,860; 6,090,555; 6,136,269; 6,022,963; 6,083,697; 6,291,183; 6,309,831; 6,416,949; 6,428,752 and 6,482,591, each of which is hereby incorporated by reference. A spotted microarray can also be used in a method according to an embodiment of the invention. An exemplary spotted microarray is a CodeLink™ Array available from Amersham Biosciences. Another microarray that is useful is one that is manufactured using inkjet printing methods such as SurePrint™ Technology available from Agilent Technologies.

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The systems and methods set forth herein can be used to detect the presence of a particular target molecule in a sample contacted with the microarray. This can be determined, for example, based on binding of a labeled target analyte to a particular probe of the microarray or due to a target-dependent modification of a particular probe to incorporate, remove, or alter a label at the probe location. Any one of several assays can be used to identify or characterize targets using a microarray as described, for example, in U.S. Patent Application Publication Nos. 2003/0108867; 2003/0108900; 2003/0170684; 2003/0207295; or 2005/0181394, each of which is hereby incorporated by reference.

Furthermore, optical systems described herein may be constructed to include various components and assemblies as described in PCT application PCT/US07/07991, entitled "System and Devices for Sequence by Synthesis Analysis", filed Mar. 30, 2007 and/or to include various components and assemblies as described in International Publication No. WO 2009/042862, entitled "Fluorescence Excitation and Detection System and Method", filed Sep. 26, 2008, both of which the complete subject matter are incorporated herein by reference in their entirety. In particular embodiments, optical systems can include various components and assemblies as described in U.S. Pat. No. 7,329,860 and WO 2009/137435, of which the complete subject matter is incorporated herein by reference in their entirety. Optical systems can also include various components and assemblies as described in U.S. patent application Ser. No. 12/638,770, filed on Dec. 15, 2009, of which the complete subject matter is incorporated herein by reference in its entirety.

In particular embodiments, methods, and optical systems described herein may be used for sequencing nucleic acids. For example, sequencing-by-synthesis (SBS) protocols are particularly applicable. In SBS, a plurality of fluorescently labeled modified nucleotides are used to sequence a plurality of clusters of amplified DNA (possibly millions of clusters) present on the surface of an optical substrate (e.g., a surface that at least partially defines a channel in a flow cell). The flow cells may contain nucleic acid samples for sequencing where the flow cells are placed within the appropriate flow cell holders. The samples for sequencing can take the form of single nucleic acid molecules that are separated from each other so as to be individually resolvable, amplified populations of nucleic acid molecules in the form of clusters or other features, or beads that are attached to one or more molecules of nucleic acid. Accordingly, sequencing can be carried out on an array such as those set forth previously herein. The nucleic acids can be prepared such that they comprise an oligonucleotide primer adjacent to an unknown target sequence. To initiate the first SBS sequencing cycle, one or more differently labeled nucleotides, and DNA polymerase, etc., can be flowed into/through the flow cell by a fluid flow subsystem (not shown). Either a single type of nucleotide can be added at a time, or the nucleotides used in the sequencing procedure can be specially designed to possess a reversible termination property, thus allowing each cycle of the sequencing reaction to occur simultaneously in the presence of several types of labeled nucleotides (e.g. A, C, T, G). The nucleotides can include detectable label moieties such as fluorophores. Where the four nucleotides are mixed together, the polymerase is able to select the correct base to incorporate and each sequence is extended by a single base. Nonincorporated nucleotides can be washed away by flowing a wash solution through the flow cell. One or more lasers may excite the nucleic acids and induce fluorescence. The fluorescence emitted from the nucleic acids is based upon the fluorophores of the incorporated



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base, and different fluorophores may emit different wavelengths of emission light. A deblocking reagent can be added to the flow cell to remove reversible terminator groups from the DNA strands that were extended and detected. The deblocking reagent can then be washed away by flowing a wash solution through the flow cell. The flow cell is then ready for a further cycle of sequencing starting with introduction of a labeled nucleotide as set forth above. The fluidic and detection steps can be repeated several times to complete a sequencing run. Exemplary sequencing methods are described, for example, in Bentley et al., *Nature* 456:53-59 (2008), WO 04/018497; U.S. Pat. No. 7,057,026; WO 91/06678; WO 07/123744; U.S. Pat. Nos. 7,329,492; 7,211,414; 7,315,019; 7,405,281, and US 2008/0108082, each of which is incorporated herein by reference.

In some embodiments, nucleic acids can be attached to a surface and amplified prior to or during sequencing. For example, amplification can be carried out using bridge amplification to form nucleic acid clusters on a surface. Useful bridge amplification methods are described, for example, in U.S. Pat. No. 5,641,658; U.S. Patent Publ. No. 2002/0055100; U.S. Pat. No. 7,115,400; U.S. Patent Publ. No. 2004/0096853; U.S. Patent Publ. No. 2004/0002090; U.S. Patent Publ. No. 2007/0128624; and U.S. Patent Publ. No. 2008/0009420. Another useful method for amplifying nucleic acids on a surface is rolling circle amplification (RCA), for example, as described in Lizardi et al., *Nat. Genet.* 19:225-232 (1998) and US 2007/0099208 A1, each of which is incorporated herein by reference. Emulsion PCR on beads can also be used, for example as described in Dressman et al., *Proc. Natl. Acad. Sci. USA* 100:8817-8822 (2003), WO 05/010145, or U.S. Patent Publ. Nos. 2005/0130173 or 2005/0064460, each of which is incorporated herein by reference in its entirety.

Other sequencing techniques that are applicable for use of the methods and systems set forth herein are pyrosequencing, nanopore sequencing, and sequencing by ligation. Exemplary pyrosequencing techniques and samples that are particularly useful are described in U.S. Pat. Nos. 6,210,891; 6,258,568; 6,274,320 and Ronaghi, *Genome Research* 11:3-11 (2001), each of which is incorporated herein by reference. Exemplary nanopore techniques and samples that are also useful are described in Deamer et al., *Acc. Chem. Res.* 35:817-825 (2002); Li et al., *Nat. Mater.* 2:611-615 (2003); Soni et al., *Clin Chem.* 53:1996-2001 (2007) Healy et al., *Nanomed.* 2:459-481 (2007) and Cockroft et al., *J. am. Chem. Soc.* 130:818-820; and U.S. Pat. No. 7,001,792, each of which is incorporated herein by reference. In particular, these methods utilize repeated steps of reagent delivery. An instrument or method set forth herein can be configured with reservoirs, valves, fluidic lines and other fluidic components along with control systems for those components in order to introduce reagents and detect optical signals according to a desired protocol such as those set forth in the references cited above. Any of a variety of samples can be used in these systems such as substrates having beads generated by emulsion PCR, substrates having zero-mode waveguides, substrates having integrated CMOS detectors, substrates having biological nanopores in lipid bilayers, solid-state substrates having synthetic nanopores, and others known in the art. Such samples are described in the context of various sequencing techniques in the references cited above and further in US 2005/0042648; US 2005/0079510; US 2005/0130173; and WO 05/010145, each of which is incorporated herein by reference.

Exemplary labels that can be detected in accordance with various embodiments, for example, when present on or

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within a support structure include, but are not limited to, a chromophore; luminophore; fluorophore; optically encoded nanoparticles; particles encoded with a diffraction-grating; electrochemiluminescent label such as Ru(bpy)<sup>32+</sup>; or moiety that can be detected based on an optical characteristic. Fluorophores that may be useful include, for example, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malachite green, Cy3, Cy5, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, alexa dyes, phycoerythrin, bodipy, and others known in the art such as those described in Haugland, *Molecular Probes Handbook*, (Eugene, OR) 6th Edition; The Synthesgen catalog (Houston, TX.), Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Ed., Plenum Press New York (1999), or WO 98/59066, each of which is hereby incorporated by reference. In some embodiments, the one pair of labels may be excitable by a first excitation wavelength and another pair of labels may be excitable by a second excitation wavelength.

Although embodiments are exemplified with regard to detection of samples that include biological or chemical substances supported by an optical substrate, it will be understood that other samples can be imaged by the embodiments described herein. Other exemplary samples include, but are not limited to, biological specimens such as cells or tissues, electronic chips such as those used in computer processors, and the like. Examples of some of the applications include microscopy, satellite scanners, high-resolution reprographics, fluorescent image acquisition, analyzing and sequencing of nucleic acids, DNA sequencing, sequencing-by-synthesis, imaging of microarrays, imaging of holographically encoded microparticles and the like.

FIG. 1 is a block diagram of an assay system 100 for biological or chemical analysis formed in accordance with one embodiment. In some embodiments, the assay system 100 is a workstation that may be similar to a bench-top device or desktop computer. For example, at least a majority of the systems and components for conducting the desired reactions can be within a common housing 117 of the assay system 100. In other embodiments, the assay system 100 includes one or more components, assemblies, or systems that are remotely located from the assay system 100 (e.g., a remote database). The assay system 100 may include various components, assemblies, and systems (or sub-systems) that interact with each other to perform one or more predetermined methods or assay protocols for biological or chemical analysis.

For example, the assay system 100 includes a system controller 102 that may communicate with the various components, assemblies, and systems (or sub-systems) of the assay system 100. As shown, the assay system 100 has an optical assembly 104, an excitation source assembly 106, a detector assembly 108, and a fluidic device holder 110 that supports one or more fluidic devices 112 having a sample thereon. The fluidic device may be a flow cell, such as the flow cell 200 described below, or the fluidic device 112 may be the fluidic device 300 described below.

In some embodiments, the optical assembly 104 is configured to direct incident light from the excitation source assembly 106 onto the fluidic device(s) 112. The excitation source assembly 106 may include one or more excitation light sources that are configured to excite labels associated with the sample. The excitation source assembly 106 may also be configured to provide incident light that is reflected and/or refracted by the samples. As shown, the samples may provide optical signals that include light emissions 116



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and/or transmission light **118**. The device holder **110** and the optical assembly **104** may be moved relative to each other. In some embodiments, the device holder **110** includes a motor assembly **132** that moves the fluidic device **112** with respect to the optical assembly **104**. In other embodiments, the optical assembly **104** may be moved in addition to or alternatively to the device holder **110**. The optical assembly **104** may also be configured to direct the light emissions **116** and/or transmission light **118** to the detector assembly **108**. The detector assembly **108** may include one or more imaging detectors. The imaging detectors may be, by way of example only, CCD or CMOS cameras, or photomultiplier tubes.

Also shown, the assay system **100** may include a fluidic control system **134** to control the flow of fluid throughout a fluidic network **135** (indicated by the solid lines) of the assay system **100**. The fluidic control system **134** may deliver reaction components (e.g., reagents) or other fluids to the fluidic device **112** during, for example, a sequencing protocol. The assay system **100** may also include a fluid storage system **136** that is configured to hold fluids that may be used by the assay system **100** and a temperature control system **138** that regulates the temperature of the fluid. The temperature control system **138** may also generally regulate a temperature of the assay system **100** using, for example, thermal modules, heat sinks, and blowers.

Also shown, the assay system **100** may include a user interface **140** that interacts with the user. For example, the user interface **140** may include a display **142** to display or request information from a user and a user input device **144** to receive user inputs. In some embodiments, the display **142** and the user input device **144** are the same device (e.g., touchscreen). As will be discussed in greater detail below, the assay system **100** may communicate with various components to perform the desired reactions. The assay system **100** may also be configured to analyze the detection data to provide a user with desired information.

The fluidic control system **134** is configured to direct and regulate the flow of one or more fluids through the fluidic network **135**. The fluidic control system **134** may include, for example, pumps and valves that are selectively operable for controlling fluid flow. The fluidic network **135** may be in fluid communication with the fluidic device **112** and the fluid storage system **136**. For example, select fluids may be drawn from the fluid storage system **136** and directed to the fluidic device **112** in a controlled manner, or the fluids may be drawn from the fluidic device **112** and directed toward, for example, a waste reservoir in the fluid storage system **136**. Although not shown, the fluidic control system **134** may also include flow sensors that detect a flow rate or pressure of the fluids within the fluidic network. The sensors may communicate with the system controller **102**.

The temperature control system **138** is configured to regulate the temperature of fluids at different regions of the fluidic network **135**, the fluid storage system **136**, and/or the fluidic device **112**. For example, the temperature control system **138** may include a thermocycler **113** that interfaces with the fluidic device **112** and controls the temperature of the fluid that flows along the fluidic device **112**. Although not shown, the temperature control system **138** may include sensors to detect the temperature of the fluid or other components. The sensors may communicate with the system controller **102**.

The fluid storage system **136** is in fluid communication with the fluidic device **112** and may store various reaction components or reactants that are used to conduct the desired reactions therein. The fluid storage system **136** may store

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fluids for washing or cleaning the fluidic network **135** or the fluidic device **112** and also for diluting the reactants. For example, the fluid storage system **136** may include various reservoirs to store reagents, enzymes, other biomolecules, buffer solutions, aqueous, and non-polar solutions, and the like. Furthermore, the fluid storage system **136** may also include waste reservoirs for receiving waste products.

The device holder **110** is configured to engage one or more fluidic devices **112**, for example, in at least one of a mechanical, electrical, and fluidic manner. The device holder **110** may hold the fluidic device(s) **112** in a desired orientation to facilitate the flow of fluid through the fluidic device **112** and/or imaging of the fluidic device **112**.

The system controller **102** may include any processor-based or microprocessor-based system, including systems using microcontrollers, reduced instruction set computers (RISC), application specific integrated circuits (ASICs), field programmable gate array (FPGAs), logic circuits, and any other circuit or processor capable of executing functions described herein. The above examples are exemplary only, and are thus not necessarily intended to limit the definition and/or meaning of the term system controller. In the exemplary embodiment, the system controller **102** executes a set of instructions that are stored in one or more storage elements, memories, or modules in order to at least one of obtain and analyze detection data. Storage elements may be in the form of information sources or physical memory elements within the assay system **100**.

The set of instructions may include various commands that instruct the assay system **100** to perform specific operations such as the methods and processes of the various embodiments described herein. The set of instructions may be in the form of a software program. As used herein, the terms “software” and “firmware” are interchangeable, and include any computer program stored in memory for execution by a computer, including RAM memory, ROM memory, EPROM memory, EEPROM memory, and non-volatile RAM (NVRAM) memory. The above memory types are exemplary only, and are thus not limiting as to the types of memory usable for storage of a computer program.

The software may be in various forms such as system software or application software. Further, the software may be in the form of a collection of separate programs, or a program module within a larger program or a portion of a program module. The software also may include modular programming in the form of object-oriented programming. After obtaining the detection data, the detection data may be automatically processed by the assay system **100**, processed in response to user inputs, or processed in response to a request made by another processing machine (e.g., a remote request through a communication link).

The system controller **102** may be connected to the other components or sub-systems of the assay system **100** via communication links (indicated by dashed lines). The system controller **102** may also be communicatively connected to off-site systems or servers. The communication links may be hardwired or wireless. The system controller **102** may receive user inputs or commands, from the user interface **140**. The user input device **144** may include a keyboard, mouse, a touch-screen panel, and/or a voice recognition system, and the like. Alternatively or in addition, the user input device **144** may also be the display **142**.

FIG. 1 also illustrates a block diagram of the system controller **102**. In one embodiment, the system controller **102** includes one or more processors or modules that can communicate with one another. The system controller **102** is illustrated conceptually as a collection of modules, but may



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be implemented utilizing any combination of dedicated hardware boards, DSPs, processors, etc. Alternatively, the system controller **102** may be implemented utilizing an off-the-shelf PC with a single processor or multiple processors, with the functional operations distributed between the processors. As a further option, the modules described below may be implemented utilizing a hybrid configuration in which certain modular functions are performed utilizing dedicated hardware, while the remaining modular functions are performed utilizing an off-the-shelf PC and the like. The modules also may be implemented as software modules within a processing unit.

The system controller **102** may include a plurality of modules **151-158** that communicate with a system control module **150**. The system control module **150** may communicate with the user interface **140**. Although the modules **151-158** are shown as communicating directly with the system control module **150**, the modules **151-158** may also communicate directly with each other, the user interface **140**, or the other systems. Also, the modules **151-158** may communicate with the system control module **150** through the other modules.

The plurality of modules **151-158** include system modules **151-153** that communicate with the sub-systems. The fluidic control module **151** may communicate with the fluidic control system **134** to control the valves and flow sensors of the fluidic network **135** for controlling the flow of one or more fluids through the fluidic network **135**. The fluid storage module **152** may notify the user when fluids are low or when the waste reservoir must be replaced. The fluid storage module **152** may also communicate with the temperature control module **153** so that the fluids may be stored at a desired temperature.

The plurality of modules **151-158** may also include an image analysis module **158** that receives and analyzes the detection data (e.g., image data) from the detector assembly **108**. The processed detection data may be stored for subsequent analysis or may be transmitted to the user interface **140** to display desired information to the user. Protocol modules **155-157** communicate with the system control module **150** to control the operation of the sub-systems when conducting predetermined assay protocols. The protocol modules **155-157** may include sets of instructions for instructing the assay system **100** to perform specific operations pursuant to predetermined protocols.

The protocol module **155** may be configured to issue commands for generating a sample within the fluidic device **112**. For example, the protocol module **155** may direct the fluid storage system **136** and the temperature control system **138** to generate the sample in a sample area. In one particular embodiment, the protocol module **155** may issue commands to perform bridge PCR where clusters of clonal amplicons are formed on localized areas within a channel (or lane) of a flow cell.

The protocol module **156** may be a sequencing-by-synthesis (SBS) module configured to issue various commands for performing sequencing-by-synthesis processes. In some embodiments, the SBS module **156** may also process detection data. After generating the amplicons through bridge PCR, the SBS module **156** may provide instructions to linearize or denature the amplicons to make sstDNA and to add a sequencing primer such that the sequencing primer may be hybridized to a universal sequence that flanks a region of interest. Each sequencing cycle extends the sstDNA by a single base and is accomplished by modified DNA polymerase and a mixture of four types of nucleotides delivery of which can be instructed by the SBS module **156**.

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The different types of nucleotides have unique fluorescent labels, and each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in each cycle. After a single base is added to the sstDNA, the SBS module **156** may instruct a wash step to remove nonincorporated nucleotides by flowing a wash solution through the flow cell. The SBS module **156** may further instruct the excitation source assembly and detector assembly to perform an image session(s) to detect the fluorescence in each of the four channels (i.e., one for each fluorescent label). After imaging, the SBS module **156** may instruct delivery of a deblocking reagent to chemically cleave the fluorescent label and the terminator from the sstDNA. The SBS module **156** may instruct a wash step to remove the deblocking reagent and products of the deblocking reaction. Another similar sequencing cycle may follow. In such a sequencing protocol, the SBS module **156** may instruct the fluidic control system **134** to direct a flow of reagent and enzyme solutions through the fluidic device **112**.

In some embodiments, the SBS module **157** may be configured to issue various commands for performing the steps of a pyrosequencing protocol. Pyrosequencing detects the release of inorganic pyrophosphate (PPi) as particular nucleotides are incorporated into the nascent strand (Ronaghi, M. et al. (1996) "Real-time DNA sequencing using detection of pyrophosphate release." *Analytical Biochemistry* 242(1), 84-9; Ronaghi, M. (2001) "Pyrosequencing sheds light on DNA sequencing." *Genome Res.* 11(1), 3-11; Ronaghi, M. et al. (1998) "A sequencing method based on real-time pyrophosphate." *Science* 281(5375), 363; U.S. Pat. Nos. 6,210,891; 6,258,568 and 6,274,320, the disclosures of which are incorporated herein by reference in their entireties. In pyrosequencing, released PPi can be detected by being immediately converted to adenosine triphosphate (ATP) by ATP sulfurylase, and the level of ATP generated is detected via luciferase-produced photons. In this case, the fluidic device **112** may include millions of wells where each well has a single capture bead having clonally amplified sstDNA thereon. Each well may also include other smaller beads that, for example, may carry immobilized enzymes (e.g., ATP sulfurylase and luciferase) or facilitate holding the capture bead in the well. The SBS module **157** may be configured to issue commands to the fluidic control module **151** to run consecutive cycles of fluids that carry a single type of nucleotide (e.g., 1st cycle: A; 2nd cycle: G; 3rd cycle: C; 4th cycle: T; 5th cycle: A; 6th cycle: G; 7th cycle: C; 8th cycle: T; and on). When a nucleotide is incorporated into the DNA, pyrophosphate is released thereby instigating a chain reaction where a burst of light is generated. The burst of light may be detected by a sample detector of the detector assembly. Detection data may be communicated to the system control module **150**, the image analysis module **158**, and/or the SBS module **157** for processing. The detection data may be stored for later analysis or may be analyzed by the system controller **102** and an image may be sent to the user interface **140**.

In some embodiments, the user may provide user inputs through the user interface **140** to select an assay protocol to be run by the assay system **100**. In other embodiments, the assay system **100** may automatically detect the type of fluidic device **112** that has been inserted into the device holder **110** and confirm with the user the assay protocol to be run. Alternatively, the assay system **100** may offer a limited number of assay protocols that could be run with the determined type of fluidic device **112**. The user may select



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the desired assay protocol, and the assay system **100** may then perform the selected assay protocol based on preprogrammed instructions.

FIGS. **2** and **3** illustrate a workstation **160** formed in accordance with one embodiment that is configured for biological and chemical analysis of a sample. As shown, the workstation **160** is oriented with respect to mutually perpendicular X, Y, and Z-axes. In the illustrated embodiment, a gravitational force *g* extends parallel to the Z-axis. The workstation **160** may include a workstation casing **162** (or workstation housing) that is shown in phantom in FIGS. **2** and **3**. The casing **162** is configured to hold the various elements of the workstation **160**. For example, the workstation **160** may include similar elements as described above with respect to the assay system **100** (FIG. **1**). As shown, the workstation **160** has an optical deck **164** having a plurality of optical components mounted thereto. The optical components may be part of an optical assembly, such as the optical assembly **602** described with reference to FIG. **38** et al. The optical deck **164** may have a fixed position with respect to the casing **162**.

The workstation **160** may also include a sample deck **166** that is movably coupled to the optical deck **164**. The sample deck **166** may have a slidable platform **168** that supports a fluidic device thereon having a sample-of-interest. In the illustrated embodiment, the fluidic device is the fluidic device **300** that is described in greater detail below. The platform **168** is configured to slide with respect to the optical deck **166** and, more specifically, with respect to an imaging lens of the optical assembly **602**. To this end, the platform **168** may slide bi-directionally along the X-axis so that the fluidic device **300** may be positioned on the sample deck **166** and so that the imaging lens may slide over the fluidic device **300** to image the sample therein. In other embodiments, the platform **168** may be stationary and the sample deck **166** may slide bi-directionally along the X-axis to position the fluidic device **300** with respect to an imaging lens of the optical assembly **602**. Thus, the platform and sample deck can be moveable relative to each other due to movement of the sample deck, platform, or both.

Also shown, the workstation **160** may include a user interface **172**, a computing system **174** (FIG. **2**), and fluid storage units **176** and **178** (FIG. **4**). The user interface **172** may be a touchscreen that is configured to display information to a user and also receive user inputs. For example, the touchscreen may receive commands to perform predetermined assay protocols or receive inquiries from the user. The computing system **174** may include processors and modules, such as the system controller **102** and the modules **151-158** described with reference to FIG. **1**. The fluid storage units **176** and **178** may be part of a larger fluid storage system. The fluid storage unit **176** may be for collecting waste that results from performing the assays and the fluid storage unit **178** may include a buffer solution.

FIG. **4** is a diagram of a fluidic network **552** that may be used in the workstation **160** (FIG. **2**). As used herein, fluids may be liquids, gels, gases, or a mixture of thereof. Also, a fluid can be a mixture of two or more fluids. The fluidic network **552** may include a plurality of fluidic components (e.g., fluid lines, pumps, flow cells or other fluidic devices, manifolds, reservoirs) configured to have one or more fluids flowing therethrough. As shown, the fluidic network **552** includes a plurality of fluidic components **553-561** interconnected through fluid lines (indicated by the solid lines). In the illustrated embodiment, the fluidic network **552** includes a buffer solution container **553**, a reagent tray **554**, a multi-port valve **555**, a bypass valve **556**, a flow rate sensor

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**557**, a flow cell **558**, another flow rate sensor **559**, a pump **560**, and a waste reservoir **561**. Fluid flow directions are indicated by arrows along the fluid lines. In addition to the fluidic components **553-561**, the fluidic network may also include other fluidic components.

The reagent tray **554** may be similar to the reaction component tray (or reaction component storage unit) **1020** described in greater detail below. The tray **1020** may include various containers (e.g., vials or tubes) containing reaction components for performing assays with embodiments described herein. Operation of the multi-port valve **555** may be controlled by an assay system, such as the assay system **100**, to selectively flow different fluids, including mixtures thereof, to the flow cell **558**. The flow cell **558** may be the flow cell **200** or the fluidic device **300**, which are described in greater detail below, or other suitable fluidic devices.

FIGS. **5-60**, which are described in greater detail below, illustrate various elements (e.g., components, devices, assemblies, systems, and the like) and methods that may be used with the workstation **160**. These elements may cooperate with one another in imaging a sample, analyzing the detection data, and providing information to a user of the workstation **160**. However, the following elements and methods may also be used independently, in other apparatuses, or with other apparatuses. For example, the flow cell **200** and the fluidic device **300** may be used in other assay systems. The optical assembly **602** (and elements thereof) may be used for examining other items, such as microcircuits. Furthermore, the device holder **400** may be used to hold other fluidic devices, such as lab-on-chip devices. Assay systems with these devices may or may not include an optical assembly to detect the desired reactions.

FIGS. **5-7** illustrate a flow cell **200** formed in accordance with one embodiment. As shown in FIGS. **5-7**, the flow cell **200** is oriented relative to the X, Y, and Z-axes. The flow cell **200** is configured to hold a sample-of-interest **205** in a flow channel **206**. The sample **205** is illustrated as a plurality of DNA clusters that can be imaged during a SBS protocol, but other samples may be used in alternative embodiments. Although only the single U-shaped flow channel **206** is illustrated, alternative embodiments may include flow cells having multiple flow channels with differently shaped paths. The flow cell **200** may be in fluid communication with a fluidic system (not shown) that is configured to deliver reagents to the sample **205** in the flow channel **206**. In some embodiments, the sample **205** may provide detectable characteristics (e.g., through fluorescence or chemiluminescence) after desired reactions occur. For instance, the flow cell **200** may have one or more sample areas or regions (i.e., areas or regions where the sample **205** is located) from which optical signals are emitted. In some embodiments, the flow cell **200** may also be used to generate the sample **205** for performing a biological or chemical assay. For example, the flow cell **200** may be used to generate the clusters of DNA before the SBS protocol is performed.

As shown in FIGS. **5-7**, the flow cell **200** can include a first layer **202** and a second layer **204** that are secured together and define the flow channel **206** therebetween. The first layer **202** has a mounting surface **208** and an outer or exterior surface **210** (FIGS. **5** and **6**). The mounting and outer surfaces **208** and **210** face in opposite directions along the Z-axis and define a thickness  $T_1$  (FIGS. **5** and **6**) therebetween. The thickness  $T_1$  is substantially uniform along an XY-plane, but may vary in alternative embodiments. The second layer **204** has a channel surface **212** (FIG. **6**) and an outer or exterior surface **214**. The channel and



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outer surfaces **212** and **214** face in opposite directions along the Z-axis and define a thickness  $T_2$  (FIG. 6) therebetween.

Also shown in FIG. 5, the first layer **202** has a dimension or length  $L_1$  measured along the X-axis and another dimension or width  $W_1$  measured along the Y-axis. In some embodiments, the flow cell **200** may be characterized as a microdevice. Microdevices may be difficult to hold or move by an individual's hands. For example, the length  $L_1$  of the flow cell **200** may be about 100 mm, or about 50 mm, or less. In particular embodiments, the length  $L_1$  is about 30 mm or less. In some embodiments, the width  $W_1$  may be about 35 mm, or about 25 mm or less or, more particularly, the width  $W_1$  may be about 15 mm or less. Furthermore, a combined or total height  $H_T$  shown in FIG. 7 (e.g., a sum of thicknesses  $T_1$  and  $T_2$ ) may be about 10 mm, or about 5 mm or less. More specifically, the height  $H_T$  may be about 2 mm or about 1.5 mm or less.

The flow cell **200** includes edges **231-234** that are linear in the illustrated embodiment. Edges **231** and **233** are spaced apart by the width  $W_1$  and extend the length  $L_1$  of the flow cell **200**. Edges **232** and **234** are spaced apart by the length  $L_1$  and extend along the width  $W_1$ . Also shown, the second layer **204** may have a dimension or length  $L_2$  measured along the X-axis and another dimension or width  $W_2$  measured along the Y-axis. In the illustrated embodiment, the edges **231-234** define a perimeter of the flow cell **200** and extend along a common cell plane that extends parallel to the XY-plane. Also shown, the second layer **204** may have edges **241-244** that are similarly oriented as the edges **231-234** as shown in FIG. 5.

In the illustrated embodiment, the width  $W_1$  is substantially greater than the width  $W_2$ , and the second layer **204** is positioned on only a portion of the mounting surface **208**. As such, the mounting surface **208** includes exposed grip portions **208A** and **208B** on opposite sides of the second layer **204**. The width  $W_2$  extends between the grip portions **208A** and **208B**. The flow cell **200** may also have cell sides **256** and **258** that face in opposite directions along the Z-axis. In the illustrated embodiment, the cell side **256** includes the grip portions **208A** and **208B** and the exterior surface **214**, and the cell side **258** includes the exterior surface **210**. Also shown, the flow cell **200** may extend lengthwise between opposite first and second cell ends **246** and **248**. In the illustrated embodiment, the edges **232** and **242** are substantially flush with respect to each other at the first cell end **246**, and the edges **234** and **244** are substantially flush with respect to each other at the opposite second cell end **248**.

As shown in FIG. 6, the second layer **204** has at least one grooved portion **216** that extends along the channel surface **212**. In the illustrated embodiment, the channel surface **212** is etched to form the grooved portion **216**, but the grooved portion **216** may be formed by other processes, such as machining the channel surface **212**. To assemble the flow cell **200**, the channel surface **212** of the second layer **204** is mounted onto and secured to the mounting surface **208** of the first layer **202**. For example, the channel and mounting surfaces **212** and **208** may be bonded together using an adhesive (e.g., light-activated resin) that prevents leakage from the flow cell **200**. In other embodiments, the channel and mounting surfaces **212** and **208** may be secured together by other adhesives or mechanically interlocked and/or held together. Thus, the first layer **202** is configured to cover the grooved portion **216** of the second layer **204** to form the flow channel **206**. In the illustrated embodiment, the grooved portion **216** may be a single continuous groove that extends substantially the length  $L_2$  toward the first end, curves, and

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then extends substantially the length  $L_2$  toward the second end. Thus, the flow channel **206** may be substantially U-shaped.

In FIGS. 5-7 the sample **205** is shown as being located along only the mounting surface **208**. However, in other embodiments, the sample **205** may be located on any surface that defines the flow channel **206**. For instance, the sample **205** may also be located on the mating surface **212** of the grooved portion **216** that partially defines the flow channel **206**.

In the illustrated embodiment, the flow channel **206** may include a plurality of channel segments **250-252**. Different channel segments may have different dimensions with respect to the immediately upstream or downstream channel segment. In the illustrated embodiment, the flow channel **206** may include a channel segment **250**, which may also be referred to as the imaging segment **250**. The channel segment **250** may have a sample area that is configured to be imaged by an imaging system (not shown). The flow channel **206** may also have channel segments **251** and **252**, which may also be referred to as non-imaging segments **250** and **252**. As shown, the channel segments **250** and **252** extend parallel to each other through the flow cell **200**. The channel segments **251** and **252** of the flow channel **206** may be sized and shaped relative to the channel segment **250** to control the flow of fluid and gases that may flow therethrough.

For example, FIG. 7 also illustrates cross-sections  $C_1$ - $C_3$  of the channel segments **250-252**, respectively, that are taken perpendicular to a flow direction  $F_1$ . In some embodiments, the cross-sections  $C_1$ - $C_3$  may be differently sized (i.e., different cross-sectional areas) to control the flow of fluid through the flow channel **206**. For example, the cross-section  $C_1$  is greater in size than the cross-sections  $C_2$  and  $C_3$ . More specifically, the channel segments **250-252** of the flow channel **206** may have a substantially equal height  $H_1$  measured between the grooved portion **216** of the channel surface **212** (FIG. 6) and the mounting surface **208**. However, the channel segments **250-252** of the flow channel **206** may have different widths  $W_3$ - $W_5$ , respectively. The width  $W_3$  is greater than the widths  $W_4$  and  $W_5$ . The channel segment **251** may constitute a curved or elbow segment that fluidically joins the channel segments **250** and **252**. The cross-section  $C_3$  is smaller than the cross-sections  $C_1$  and  $C_2$ . For example, the width  $W_5$  is less than the widths  $W_3$  and  $W_4$ .

FIG. 8 is an enlarged view of the curved segment **251** and portions of the channel segments **250** and **252**. As described above, the channel segments **250** and **252** may extend parallel to each other. Within the flow channel **206**, it may be desirable to have a uniform flow across the sample area. For example, the fluid may include stream portions  $F_2$ - $F_4$ . Dimensions of the channel segments **250-252** may be configured so that the stream portions  $F_2$ - $F_4$  have substantially equal flow rates across the sample area. In such embodiments, different sections or portions of the sample **205** (FIG. 5) may have a substantially equal amount of time to react with reaction components within the fluid.

To this end, the curved segment **251** of the flow channel **206** may have a non-continuous contour that fluidically joins the channel segments **250** and **252**. For example, as shown in FIG. 8, the curved segment **251** may include a tapering portion **270**, an intermediate portion **276**, and a downstream portion **278**. As shown, the tapering portion **270** has a width  $W_{5,A}$  that gradually reduces in size. More specifically, the curved segment **251** may include sidewalls **272** and **274** that extend inward toward each other at a substantially equal angle. The intermediate portion **276** curves from the tapering



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portion 270 to the downstream portion 278. The intermediate portion 276 has a width  $W_{5B}$  that reduces in size and then begins to increase in size. The downstream portion 278 has a substantially uniform width  $W_{5C}$  throughout and extends along a substantially linear path from the intermediate portion 276 to the channel segment 252. In other words, the sidewalls 272 and 274 may extend parallel to each other throughout the downstream portion 278.

Returning to FIG. 7, the flow cell 200 includes inlet and outlet ports 222 and 224, respectively. The inlet and outlet ports 222 and 224 are formed only through the second layer 204. However, in alternative embodiments, the inlet and outlet ports 222 and 224 may be formed through only the first layer 202 or through both layers 202 and 204. The flow channel 206 is in fluid communication with and extends between the inlet and outlet ports 222 and 224. In particular embodiments, the inlet and outlet ports 222 and 224 are located proximate to each other at the cell end 248 of the flow cell 200 (or proximate to the edges 234 and 244). For example, a spacing 282 that separates the inlet and outlet ports 222 and 224 may be approximately equal to the width  $W_3$ . More specifically, the spacing 282 may be about 3 mm, about 2 mm, or less. Furthermore, the channel segments 250 and 252 may be separated by a spacing 280. The spacing 280 may be less than the width  $W_3$  of the channel segment 250 or, more particularly, less than the width  $W_4$  of the channel segment 252. Thus, a path of the flow channel 206 may be substantially U-shaped and, in the illustrated embodiment, have a non-continuous contour along the curved segment 251.

In alternative embodiments, the flow channel 206 may have various paths such that the inlet and outlet ports 222 and 224 have different locations in the flow cell 200. For example, the flow channel may form a single lane that extends from the inlet port at one end of the flow cell to the outlet port at the opposite end of the flow cell.

With respect to FIG. 6, in some embodiments, the thickness  $T_2$  (FIG. 6) of the second layer 204 is substantially uniform along the imaging portion 250. The uniform thickness  $T_2$  along the imaging portion 250 may be configured to transmit optical signals therethrough. Furthermore, the thickness  $T_1$  of the first layer 202 is substantially uniform along the imaging portion 250 and configured to permit uniform transfer of thermal energy therethrough into the flow channel 206.

FIGS. 9-11 illustrate a fluidic device 300 formed in accordance with one embodiment. For illustrative purposes, the fluidic device 300 is oriented with respect to the mutually perpendicular X, Y, and Z-axes shown in FIGS. 9 and 10. FIGS. 9 and 10 are perspective views of the fluidic device 300. As shown in FIGS. 9 and 10, the fluidic device 300 includes a cartridge (or flow cell carrier) 302 and the flow cell 200. The cartridge 302 is configured to hold the flow cell 200 and facilitate orienting the flow cell 200 for an imaging session.

In some embodiments, the fluidic device 300 and the cartridge 302 may be removable such that the cartridge 302 may be removed from an imaging system (not shown) by an individual or machine without damage to the fluidic device 300 or cartridge 302. For example, the cartridge 302 may be configured to be repeatedly inserted and removed into the imaging system without damaging the cartridge 302 or rendering the cartridge 302 unsuitable for its intended purpose. In some embodiments, the fluidic device 300 and the cartridge 302 may be sized and shaped to be handheld by an

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individual. Furthermore, the fluidic device 300 and the cartridge 302 may be sized and shaped to be carried by an automated system.

As shown in FIGS. 9 and 10, the cartridge 302 may include a housing or carrier frame 304 and a cover member 306 that is coupled to the housing 304. The housing 304 has housing or carrier sides 303 and 305 that face in opposite directions along the Z-axis and have a height  $H_2$  (shown in FIG. 11) extending therebetween. As shown in FIG. 9, the housing 304 includes a bridge member 324 at a loading end 316 of the fluidic device 300 and a base member 326 at an opposite receiving end 318 of the fluidic device 300. The housing 304 also includes a pair of spaced apart leg extensions 328 and 330 that extend between the bridge and base members 324 and 326. The bridge member 324 extends between and joins the leg extensions 328 and 330. The bridge member 324 may include a recess 321 (shown in FIG. 10) that opens to an exterior of the fluidic device 300. As shown in FIG. 9, the leg extensions 328 and 330 may have a plurality of grip members 371-374 that are configured to grip the cell side 256 of the flow cell 200.

Also shown in FIG. 9, the fluidic device 300 may have a device window 315 that passes entirely through the cartridge 302 along the Z-axis. In the illustrated embodiment, the device window 315 is substantially framed by the bridge member 324, the cover member 306, and the leg extensions 328 and 330. The device window 315 includes a reception space 308 and a plurality of recesses 320 and 322 that are immediately adjacent to the reception space 308. The reception space 308 is configured to receive the flow cell 200. When the flow cell 200 is positioned within the reception space 308, the flow cell 200 is exposed to an exterior of the fluidic device 300 such that the flow cell 200 may be viewed or directly engaged along the housing side 303 and also the housing side 305. For example, the cell side 258 (also shown in FIG. 11) that faces in an opposite direction along the Z-axis relative to the cell side 256. The cell side 256 may be viewed by the imaging system or directly engaged by another component along the housing side 303. Likewise, the cell side 258 may be viewed by the imaging system or directly engaged by another component along the housing side 305.

With respect to FIGS. 9 and 10, the cover member 306 may include a cover body 340 and a gasket 342 that are coupled to each other. The gasket 342 includes inlet and outlet passages 346 and 344 (shown in FIG. 9) that are located proximate to one another. In the illustrated embodiment, the cover body 340 and the gasket 342 are co-molded into a unitary structure. When formed, the cover body 340 and the gasket 342 may have different compressible properties. For example, in particular embodiments, the gasket 342 may comprise a material that is more compressible than material of the cover body 340. However, in alternative embodiments, the cover body 340 and the gasket 342 may be separate parts that are coupled together (e.g., mechanically or using an adhesive). In other embodiments, the cover body 340 and the gasket 342 may be different portions or regions of a single continuous structure.

The cover member 306 may be movably coupled to the housing 304. For example, the cover member 306 may be rotatably coupled to the base member 326 of the housing 304. In such embodiments, the gasket 342 is rotatable about an axis of rotation  $R_1$  between a mounted position (shown in FIG. 9) and a disengaged position (shown in FIG. 10). In other embodiments in which the cover member 306 is movably coupled to the housing 304, the cover member 306 may be detachable from the housing 304. For example,



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when attached to the housing 304, the detachable cover member may be in a mounted position that is similar to the mounted position as shown in FIG. 9. When unattached to the housing 304, the detachable cover member may be completely removed in a disengaged position.

Also shown in FIG. 10, the housing 304 may define a cartridge cavity 338 (FIG. 10) that is accessible when the cover member 306 is in the disengaged position. In some embodiments, an identification transmitter 336 may be positioned within the cartridge cavity 338. The identification transmitter 336 is configured to communicate information about the flow cell 200 to a reader. For example, the identification transmitter 336 may be an RFID tag. The information provided by the identification transmitter 336 may, for example, identify the sample in the flow cell 200, a lot number of the flow cell or sample, a date of manufacture, and/or the assay protocol to be performed when the flow cell 200 is inserted into the imaging system. The identification transmitter 336 may communicate other information as well.

FIG. 11 is a cross-section of the fluidic device 300 viewed along the Y-axis. In some embodiments, the reception space 308 is sized and shaped relative to the flow cell 200 so that the flow cell 200 is retained in the space, but in at least some configurations may float therein. As used herein, the term “float” and like terms includes the component being permitted to move a limited distance in at least one direction (e.g., along the X, Y, or Z-axes). For example, the flow cell 200 may be capable of shifting within the reception space 308 along the XY-plane. The flow cell 200 may also be capable of moving in a direction along the Z-axis within the reception space 308. Furthermore, the flow cell 200 can also be capable of slightly rotating within the reception space 308. In particular embodiments, the housing 304 permits the flow cell 200 to shift, move, and slightly rotate within the reception space 308 with respect to any of the X, Y, and Z-axes.

In some embodiments, the reception space 308 may also be characterized as the space that the fluidic device 300 allows the flow cell 200 to move freely within when the fluidic device 300 is holding the flow cell 200. Thus, dimensions of the reception space 308 may be based upon positions of reference surfaces of the fluidic device 300 that can directly engage the flow cell 200. The reference surfaces may be surfaces of the housing 304 or the cover member 306, including the gasket 342. For example, FIG. 11 illustrates a plurality of reference surfaces 381-387. The reference surfaces 381 and 382 of the grip members 371 and 372, respectively, and the reference surface 383 of the gasket 342 may limit movement of the flow cell 200 beyond a predetermined level when the flow cell 200 is held within the reception space 308. The reference surface 384 of the gasket 342 and the reference surface 385 of the bridge member 324 may limit movement of the flow cell 200 along the XY-plane. Furthermore, the reference surfaces 386 and 387 of the bridge member 324 and the cover member 306, respectively, may also limit movement of the flow cell 200 along the Z-axis. However, the reference surfaces 381-387 are exemplary only and the fluidic device 300 may have other reference surfaces that limit movement of the flow cell 200.

To assemble the fluidic device 300, the flow cell 200 may be loaded into the reception space 308. For example, the flow cell 200 may be advanced toward the device window 315 along the housing side 305. The edge 234 (FIG. 5) may be advanced between the grip members 372 and 373 and the gasket 342. The cell side 256 may then be rotated toward the grip members 371-374 so that the grip members 371-374

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interface the cell side 256. The edge 232 (FIG. 5) may then be moved toward the bridge member 324 and, more specifically, the reference surface 385 of the bridge member 324. In some embodiments, the bridge member 324 may be deflected or bent to provide more space for positioning the cell end 246 (FIG. 5) thereon. When the flow cell 200 is loaded into the cartridge 302, the housing 304 and the cover member 306 may effectively grip the perimeter of the flow cell 200 such that the flow cell 200 is confined to move only within the reception space 308.

In alternative embodiments, the cell end 246 may be first inserted positioned by the bridge member 324 and then the gasket 342. In other embodiments, the flow cell 200 may approach the housing side 303. The grip members 371-374 may have tapered or beveled surfaces that permit the flow cell 200 to be snapped into position within the reception space 308.

Before, after, or during the loading of the flow cell 200, the cover member 306 may be moved to the disengaged position so that the identification transmitter 336 (FIG. 10) may be positioned with the cartridge cavity 338 (FIG. 10). When the gasket 342 is in the mounted position, the inlet and outlet passages 346 and 344 may have a predetermined location and orientation with respect to the housing 304 and the reception space 308. The gasket 342 may be mounted over the flow cell 200 along an exposed portion of the flow cell 200 (i.e., the cell side 256). The inlet and outlet passages 346 and 344 may be generally aligned with the inlet and outlet ports 224 and 222 (FIG. 5).

However, it should be noted that the illustrated fluidic device 300 is only one particular embodiment, and the fluidic device 300 may have different configurations in alternative embodiments. For example, in alternative embodiments, the flow cell 200 may not be exposed to the exterior of the fluidic device 300 along each of the housing sides 303 and 305. Instead, the flow cell 200 may be exposed to the exterior along only one of the housing sides (e.g., the housing side 303). Furthermore, in alternative embodiments, the cover member 306 may not be rotatably coupled to the housing 304. For example, the cover member 306 may be entirely detachable.

FIGS. 12-15 illustrate fluidic devices 900 and 920 formed in accordance with alternative embodiments that may also be used in assay systems, such as the assay system 100 (FIG. 1) and the workstation 160 (FIG. 2). The fluidic devices 900 and 920 may include similar features as the fluidic device 300. For example, as shown, in FIGS. 12 and 13, the fluidic device 900 may include a cartridge (or flow cell carrier) 902 and the flow cell 200. The cartridge 902 is configured to hold the flow cell 200 and facilitate orienting the flow cell 200 for an imaging session. The cartridge 902 includes a housing 904 and a cover member 906 that is movably mounted to the housing 904. The cover member 906 is in the mounted position in FIG. 12 and the disengaged position in FIG. 13.

Also shown in FIGS. 12 and 13, the fluidic device 900 may include a sealing member 910 that covers the inlet and outlet ports 222 and 224 (FIG. 13) of the flow cell 200. In some embodiments, the sealing member 910 is configured to facilitate retaining fluid within the flow channel 206 so that the sample 205 (FIG. 5) within the flow channel 206 remains in a fluid environment. However, in some embodiments, the sealing member 910 may be configured to prevent unwanted materials from entering the flow channel 206. As shown in FIGS. 12 and 13, the sealing member 910 is a single piece of tape that extends between the cell ends 246 and 248 (FIG. 13). An overhang portion 912 may extend away from the cell end 246. In alternative embodiments, the sealing member



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910 may be more than one piece of tape (e.g., one piece of tape for each of the inlet and outlet ports 222 and 224) or the sealing member 910 may be other elements capable of covering the inlet and outlet ports 222 and 224. For example, the sealing member 910 could include plugs.

In some embodiments, the sealing member 910 covers the inlet and outlet ports 222 and 224 when the fluidic device 900 is not mounted to an assay system. For example, the sealing member 910 may be used when the fluidic device 900 is being stored or transported, or when a sample is being grown or generated within the flow cell 200. In such instances, the sealing member 910 may be secured to the flow cell 200 and the housing 904 as shown in FIG. 13. More specifically, the sealing member 910 may couple to and extend along the cell side 256 and cover the inlet and outlet ports 222 and 224. The sealing member 910 may also couple to a base member 914 of the housing 904. The cover member 906 may then be moved to the mounted position as shown in FIG. 12 such that the sealing member 910 is sandwiched between the inlet and outlet ports 222 and 224 and the cover member 906. The cover member 906 may facilitate preventing the sealing member 910 from being inadvertently removed. In alternative embodiments, the sealing member 910 may cover inlet and outlet passages 916 and 918 of the cover member 906.

FIGS. 14 and 15 illustrate the fluidic device 920, which may also have similar features as the fluidic devices 300 and 900. As shown, the fluidic device 920 includes a cartridge (or flow cell carrier) 922 and the flow cell 200. The cartridge 922 includes a housing 924 and a cover member 925 that is movably mounted to the housing 924. The cover member 925 is only shown in the mounted position in FIGS. 14 and 15. The housing 924 and the cover member 925 may be similar to the housings 204 and 904 and the cover member 306 and 906 described above.

However, the housing 924 may also include fin projections 926 and 928. The fin projections 926 and 928 are sized and shaped to be gripped by an individual or robotic device, such as when the fluidic device 920 is being inserted in or removed from a device holder (not shown). In some embodiments, the fin projections 926 and 928 may prevent the cover assembly (not shown) from moving to the closed position if the fluidic device 920 is not properly positioned. The fin projections 926 and 928 may include tactile features 927 and 929 that are configured to be gripped by the individual. In the illustrated embodiment, the fin projections 926 and 928 are located at a receiving end 930 of the fluidic device 920. The cover member 925 may extend between the fin projections 926 and 928. However, the fin projections 926 and 928 may have other locations along the cartridge 902.

FIGS. 16-24 show various features of a fluidic device holder 400 formed in accordance with one embodiment. FIG. 16 is a partially exploded view of the holder 400. When assembled, the holder 400 may be used to hold the fluidic device 300 (FIG. 9) and the flow cell 200 (FIG. 5) in a desired orientation during an imaging session. Furthermore, the holder 400 may provide an interface between the fluidic device 300 and the imaging system (not shown) in which the holder 400 may be configured to direct fluids through the flow cell 200 and provide or remove thermal energy from the flow cell 200. Although the holder 400 is shown as holding the fluidic device 300, the holder 400 may be configured to hold other fluidic devices, such as lab-on-chip devices or flow cells without cartridges.

As shown in FIG. 16, the holder 400 may include a removable cover assembly 404 and a support structure 402. In some embodiments, the holder 400 may also include a

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plate structure 406 and a movable platform 408. The plate structure 406 is operatively coupled to the cover assembly 404 and includes an opening 410 therethrough. Likewise, the platform 408 includes an opening 412 therethrough. The support structure 402 may include a heat sink 414 and a thermal module (or thermocycler) 416 that is mounted onto the heat sink 414. The thermal module 416 includes a base portion 418 and a pedestal 420. When the holder 400 is assembled, the support structure 402, the platform 408, and the plate structure 406 are stacked with respect to each other. As such, the opening 412 is sized and shaped to receive the base portion 418, and the opening 410 is sized and shaped to receive the pedestal 420. When assembled, the cover assembly 404 may be operatively coupled to the plate structure 406 and the support structure 402.

FIG. 17 shows the assembled holder 400. In the illustrated embodiment, a panel 424 is positioned over the plate structure 406 (FIG. 16). As shown in FIGS. 16 and 17, the cover assembly 404 includes a cover housing 435 that is coupled to the plate structure 406. The cover housing 435 may be substantially U-shaped having a pair of spaced apart housing legs 436 and 438 that extend in a common direction. The housing legs 436 and 438 may be rotatably coupled to the plate structure 406 at joints 437 and 439. The cover housing 435 may also include a bridge portion 440 that extends between and joins the housing legs 436 and 438. In this manner, the cover assembly 404 may be configured to provide a viewing space 442 (FIG. 17). The viewing space 442 may be sized and shaped to permit an imaging lens (not shown) to move in a direction  $D_x$  (FIG. 17) along and over the flow cell 200.

In the illustrated embodiment, the cover assembly 404 is movable relative to the plate structure 406 or support structure 402 between an open position (shown in FIG. 16) and a closed position (shown in FIG. 17). In the open position, the cover assembly 404 is withdrawn or retracted to permit access to a loading region 422 (shown in FIG. 18) of the holder 400 so that the fluidic device 300 may be removed from or inserted into the loading region 422. In the closed position, the cover assembly 404 is mounted over the fluidic device 300. In particular embodiments, the cover assembly 404 establishes a fluid connection with the fluidic device 300 in the closed position and presses the flow cell 200 against the support structure 402.

As shown in FIG. 16, in some embodiments, the holder 400 includes a coupling mechanism 450 to facilitate holding the cover assembly 404 in the closed position. For example, the coupling mechanism 450 may include an operator-controlled element 452 that includes a button 453 that is coupled to a pair of latch openings 456 and 458. The coupling mechanism 450 also includes a pair of latch ends 454 and 455 that project away from a mating face 460 of the cover housing 435. The cover housing 435 may be biased into the open position by spring elements 464 and 466. When the cover assembly 404 is moved into the closed position by an individual or machine, the latch ends 454 and 455 are inserted into the latch openings 456 and 458, respectively, and grip the operator-controlled element 452. To move the cover assembly 404 into the open position, the individual or machine may actuate the button 453 by, for example, pushing the button 453 inward. Since the cover housing 435 is biased by the spring elements 464 and 466, the cover housing 435 is rotated away from the panel 424 (FIG. 17) about the joints 437 and 439.

In alternative embodiments, the coupling mechanism 450 may include other elements to facilitate holding the cover assembly 404 in the closed position. For example, the latch



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ends **454** and **455** may be replaced by magnetic elements or elements that form an interference fit with openings.

FIG. **18** is an isolated perspective view of thermal module **416** and the heat sink **414** of the support structure **402**. The thermal module **416** may be configured to control a temperature of the flow cell **200** for predetermined periods of time. For example, the thermal module **416** may be configured to raise the temperature of the flow cell **200** so that DNA in the sample may denature. Furthermore, the thermal module **416** may be configured to remove thermal energy thereby lowering the temperature of the flow cell **200**. As shown, the pedestal **420** includes a base surface **430** that is sized and shaped to interface with the flow cell **200** (FIG. **5**). The base surface **430** faces in a direction along the Z-axis. The pedestal **420** may also include a plurality of alignment members **431-433** that are positioned around the base surface **430**. In the illustrated embodiment, the alignment members **431-433** have fixed positions with respect to the base surface **430**. The alignment members **431-433** have corresponding reference surfaces that are configured to engage the flow cell **200** and facilitate positioning the flow cell **200** for imaging. For example, the reference surfaces of the alignment members **431-433** may face in respective directions along the XY-plane and, as such, may be configured to limit movement of the flow cell **200** along the XY-plane. The support structure **402** may include at least a portion of the loading region **422**. The loading region **422** may be partially defined by the base surface **430** and the reference surfaces of the alignment members **431-433**.

FIGS. **19** and **20** illustrate an alignment assembly **470** that may be used with the holder **400** in accordance with one embodiment. FIG. **19** is a plan view of the holder **400** in which the cover housing **435** is shown in phantom to illustrate the alignment assembly **470**. FIG. **20** is a perspective view of the holder **400** in which the cover assembly **404** is in the open position. (In both FIGS. **19** and **20**, the panel **424** (FIG. **17**) has been removed for illustrative purposes.)

The fluidic device **300** is loaded into the loading region **422** in FIGS. **19** and **20**. When the fluidic device **300** is loaded, the flow cell **200** is placed onto the base surface **430** (FIG. **18**) and the alignment members **432**, **433**, and **431** are advanced through the recesses **320**, **322**, and **321** (FIGS. **9** and **10**) of the cartridge **302**. More specifically, the device window **315** (FIG. **9**) along the housing side **305** may be sized and shaped to be greater than a perimeter of the base surface **430**. As such, the cartridge **302** or housing **304** may be allowed to fall around the base surface **430**, but the flow cell **200** is prevented from falling by the base surface **430**. In this manner, the cell side **258** of the flow cell **200** may be pressed against the base surface **430** so that the thermal module **416** may control a temperature of the flow cell **200**. When the flow cell **200** is mounted on the base surface **430**, the reference surfaces **381-383** (FIG. **11**) of the cartridge **302** are pressed against the cell side **256** (FIG. **11**). At this time, a cell plane of the flow cell **200** that extends along the sample **205** may be substantially aligned with an object plane of the imaging system.

In the illustrated embodiment, when the fluidic device **300** is loaded into the loading region **422**, an identification reader of the assay system may detect information from the identification transmitter **336** (FIG. **10**). For example, the holder **400** may include an identification reader (not shown) in the plate structure **406** proximate to the identification transmitter **336**. The identification reading may occur before the cover assembly **404** is mounted onto the fluidic device **300**.

With reference to FIGS. **19** and **20**, the alignment assembly **470** includes various elements that cooperate together in

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orienting and positioning the flow cell **200** for imaging. For example, the alignment assembly **470** includes a movable locator arm **472** and an actuator **474** that is operatively coupled to the locator arm **472**. As shown, the actuator **474** includes a lever **476** and a pin element **478** that is coupled to the cover housing **435**. In the illustrated embodiment, the lever **476** is rotatable about an axis of rotation  $R_2$  (FIG. **19**). The lever **476** may be L-shaped having a first extension **480** configured to engage the pin element **478** and a second extension **482** configured to engage the locator arm **472**. The locator arm **472** is also rotatable about an axis of rotation  $R_3$  (FIG. **19**) and includes a finger **484** having an engagement end **486**. The alignment assembly **470** also includes a biasing element **490** (e.g., a coil spring) that engages the finger **484**. The engagement end **486** is configured to engage the cartridge **302** of the fluidic device **300**. In alternative embodiments, the engagement end **486** may be configured to directly engage the flow cell **200**.

The alignment assembly **470** is in an engaged arrangement in FIG. **19** and in a withdrawn arrangement in FIG. **20**. The locator arm **472** is in a retracted position when the alignment assembly **470** is in the withdrawn arrangement and in a biased position when the alignment assembly **470** is in the engaged arrangement. To align the flow cell **200** in the loading region **422**, the alignment assembly **470** is changed from the withdrawn arrangement to the engaged arrangement. For example, when the cover housing **435** is moved to the open position shown in FIG. **20**, the pin element **478** engages the first extension **480** of the lever **476** causing the lever **476** to rotate about the axis  $R_2$  in a counter-clockwise direction (as shown in FIG. **19**). The cover housing **435** may be maintained in the open position by the spring elements **464** and **466** (FIG. **16**). When the lever **476** is rotated, the second extension **482** rotates about the axis  $R_2$  and engages the locator arm **472**. The locator arm **472** is rotated about the axis  $R_3$  in a clockwise direction (as shown in FIG. **19**). When the locator arm **472** is rotated, the locator arm **472** is moved to the retracted position. When moved to the retracted position, the engagement end **486** is moved away from the reference surfaces of the alignment members **431-433**.

To change the alignment assembly **470** from the withdrawn arrangement to the engaged arrangement, the cover housing **435** may be rotated toward the fluidic device **300** and mounted over the flow cell **200**. When the cover housing **435** is moved toward the fluidic device **300**, the pin element **478** is rotated away from the first extension **480** of the lever **476**. When the second extension **482** moves away from the locator arm **472**, potential energy stored in the biasing element **490** may cause the locator arm **472** to rotate in a counter-clockwise direction such that the engagement end **486** presses against the cartridge **302**. As such, the locator arm **472** is moved to the biased position. When moved to the biased position, the engagement end **486** is moved toward the reference surfaces of the alignment members **431-433**.

FIG. **21** is an enlarged plan view of the fluidic device **300** in the loading region **422** when the engagement end **486** of the locator arm **472** is pressed against the cartridge **302**. The engagement end **486** may be configured to move within the XY-plane between the retracted and biased positions. When the engagement end **486** is moved toward the biased position and presses against the cartridge **302**, the engagement end **486** provides a force  $F_{XY}$  against the cartridge **302**. The cartridge **302** may shift along the XY-plane and/or press the flow cell **200** against the reference surfaces of the alignment members **431-433**. The force  $F_{XY}$  has an X-component and a Y-component. The X-component may press the flow cell



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200 against the alignment member 431, and the Y-component may press the flow cell 200 against the alignment members 432 and 433. As such, the alignment member 431 may stop movement of the flow cell 200 in a direction along the X-axis, and the alignment members 432 and 433 may stop movement of the flow cell 200 in a direction along the Y-axis.

Before the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 of the cover member 306 may be approximately aligned with the inlet and outlet ports 224 and 222 (FIG. 7), respectively, of the flow cell 200. After the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 are effectively (or operatively) aligned with the inlet and outlet ports 224 and 222 so that fluid may effectively flow therethrough.

Accordingly, the cover assembly 404 may be operatively coupled to the alignment assembly 470 such that one step or action causes the alignment assembly 470 to engage the fluidic device 300. More specifically, as the cover assembly 404 is mounted over the device in the closed position, the actuator 474 moves the locator arm 472 to the biased position. In the biased position, the locator arm 472 holds the flow cell 200 against the reference surfaces of the alignment members 431-433 in a fixed position along the XY-plane. When the cover assembly 404 is in the closed position, the viewing space 442 (FIG. 17) may be located over the flow cell 200 so that an imaging lens may move along the flow cell 200 to image the flow channel 206. As the cover assembly 404 is moved to the open position, the actuator 474 moves the locator arm 472 to the retracted position. However, in the illustrated embodiment, the flow cell 200 remains in position when the locator arm 472 is retracted. Accordingly, the flow cell 200 may be floatable relative to various elements. For example, the flow cell 200 may be floatable with respect to the cover member 306 and the gasket 342 when the cover member 306 is in the mounted position. The flow cell 200 may also be floatable relative to the cover assembly 404 and the base surface 430.

In some embodiments, the alignment assembly 470 and the cover assembly 404 may operate at a predetermined sequence. For example, in particular embodiments, the locator arm 472 is configured to hold the flow cell 200 against the alignment members 431-433 in the fixed position before the cover assembly 404 reaches the closed position. When the cover assembly 404 reaches the closed position, the cover assembly 404 may facilitate pressing the flow cell 200 against the base surface 430 and also pressing the inlet and outlet passages 346 and 344 against the inlet and outlet ports 224 and 222. Generally, the alignment assembly 470 can be configured to position the flow cell 200 in the x and y dimensions after the base surface 430 positions the flow cell 200 in the z dimension. Alternatively, an alignment assembly can be configured to position the flow cell 200 first in the x and y dimensions and then in the z dimension. Thus, alignment in the x, y and z dimensions can occur sequentially and in various orders in response to a single step or motion carried out by a user.

In alternative embodiments, the alignment assembly 470 may not be operatively coupled to the cover assembly 404 as described above. Instead, the alignment assembly 470 and the cover assembly 404 may operate independently from each other. As such, an individual may be required to perform a plurality of steps to align the flow cell 200 and fluidically couple the flow cell 200. For example, the alignment assembly 470 can be separately actuated by an individual thereby moving the locator arm 472 to align the flow

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cell 200. After the flow cell 200 is aligned, the individual may then lower the cover assembly 404 onto the flow cell 200. Furthermore, the alignment assembly 470 may comprise additional and/or other components than those described above.

FIG. 22 is an isolated perspective view of the cover assembly 404 in the closed position. FIG. 22 illustrates dimensions of the viewing space 442. As shown, the cover housing 435 may have a top surface 492. The viewing space 442 may have a depth  $D_p$  that is measured from the top surface 492 to the fluidic device 300 or the flow cell 200. The viewing space 442 may also have a width  $W_g$  measured along the Y-axis and a length  $L_g$  measured along the X-axis. The dimensions of the viewing space 442 may be sized so that an imaging lens (not shown) may move therethrough over the flow cell 200. More specifically, an imaging lens may enter the viewing space 442 through an access opening 443 and move in a direction along the X-axis over the flow cell 200.

FIG. 23 is a cross-section of the cover assembly 404 taken along the line 23-23 in FIG. 22. In the illustrated embodiment, the cover assembly 404 may include a plurality of compression arms 494 and 496. The compression arms 494 and 496 are configured to provide respective compressive forces  $F_{C1}$  and  $F_{C2}$  against the housing side 303 of the fluidic device 300. In the illustrated embodiment, the compression arms 494 and 496 press against the cartridge 302. However, in alternative embodiments, the compression arms 494 and 496 may press against the flow cell 200.

The compressive forces  $F_{C1}$  and  $F_{C2}$  press the housing 304 of the fluidic device 300 thereby pressing the cell side 256 (FIG. 9) of the flow cell 200 against the thermal module 416. As such, the flow cell 200 may maintain intimate contact with the base surface 430 for transferring thermal energy therebetween. In the illustrated embodiment, the compression arms 494 and 496 operate independently of each other. For example, each of the compression arms 494 and 496 is operatively coupled to respective compression springs 495 and 497.

As shown in FIG. 23, the compression arms 494 and 496 extend toward the viewing space 442 and the loading region 422. The compression arms 494 and 496 may engage the housing side 303 when the cover assembly 404 is moved to the closed position. As the compression arms 494 and 496 press against the housing side 303, resistance from the housing side 303 may cause the compression arms 494 and 496 to rotate about axes  $R_4$  and  $R_5$ . Each of the compression springs 495 and 497 may resist the rotation of the respective compression arm thereby providing the corresponding compressive force  $F_C$  against the housing side 303. Accordingly, the compression arms 494 and 496 are independently biased relative to each other.

FIG. 24 is an isolated perspective view of a flow assembly 500 of the cover assembly 404 (FIG. 16). The flow assembly 500 includes a manifold body 502 and upstream and downstream flow lines 504 and 506. As shown in FIG. 16, the manifold body 502 may extend between the housing legs 436 and 438. Returning to FIG. 24, the flow lines 504 and 506 are mechanically and fluidically coupled to the manifold body 502 at body ports 508 and 510, respectively. The flow lines 504 and 506 also include line ends 514 and 516 that are configured to be inserted into the inlet and outlet passages 346 and 344 of the gasket 342.

As shown in FIG. 24, the flow assembly 500 is in a mounted position with respect to the gasket 342. In the mounted position, the line ends 514 and 516 are inserted into the inlet and outlet passages 346 and 344, respectively, so



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that fluid may flow through the flow cell **200**. Furthermore, in the mounted position, the flow assembly **500** may press the gasket **342** (FIG. **9**) against the flow cell **200** so that the fluid connection is effectively sealed. To this end, the flow assembly **500** may include biasing springs **520** and **522**. The biasing springs **520** and **522** are configured to press against an interior of the cover housing **435** (FIG. **16**) and provide a force  $F_{C3}$  against the gasket **342**. The coupling mechanism **450** (FIG. **16**) may facilitate maintaining the seal against the gasket **342**.

Accordingly, the cover assembly **404** may press against the housing **304** of the fluidic device **300** at three separate compression points. More specifically, the gasket **342** may constitute a first compression point  $P_1$  (shown in FIG. **24**) when engaged by the line ends **514** and **516**, and the compression arms **494** and **496** may contact the fluidic device **300** at second and third compression points  $P_2$  and  $P_3$  (shown in FIG. **23**). As shown in FIGS. **22-24**, the three compression points  $P_1$ - $P_3$  are distributed about the flow cell **200**. Moreover, the cover assembly **404** independently provides the compressive forces  $F_{C1}$ - $F_{C3}$  at the compression points  $P_1$ - $P_3$ . As such, the cover assembly **404** may be configured to provide a substantially uniform compressive force against the fluidic device **300** so that the flow cell **200** is uniformly pressed against the base surface **430** and the fluidic connection is sealed from leakage.

FIG. **25** is a block diagram of a method **530** of positioning a fluidic device for sample analysis. The method **530** includes positioning at **532** a removable fluidic device on a base surface. The fluidic device may be similar to the fluidic device **300** described above. For example, the fluidic device may include a reception space, a flow cell located within the reception space, and a gasket. The flow cell may extend along an object plane in the reception space and be floatable relative to the gasket within the object plane. The method **530** also includes moving the flow cell at **534** within the reception space while on the base surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket. The moving operation **534** may include actuating a locator arm to press the flow cell against alignment members.

FIG. **26** is a block diagram illustrating a method **540** of positioning a fluidic device for sample analysis. The fluidic device **300** may be similar to the fluidic device **300** described above. The method **540** includes providing a fluidic device at **542** having a device housing that includes a reception space and a floatable flow cell located within the reception space. The device housing may include recesses that are located immediately adjacent to the reception space. The method also includes positioning at **544** the fluidic device on a support structure having alignment members. The alignment members may be inserted through corresponding recesses. Furthermore, the method **540** may include moving the flow cell at **546** within the reception space. When the flow cell is moved within the reception space, the alignment members may engage edges of the flow cell. The moving operation **546** may include actuating a locator arm to press the flow cell against the alignment members.

FIG. **27** is a block diagram illustrating a method **550** for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes. The method **550** includes providing an alignment assembly at **552**. The alignment assembly may be similar to the alignment assembly **470** described above. More specifically, the alignment assembly may include a movable locator arm that has an engagement end. The locator arm may be movable between retracted and

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biased positions. The method **550** also includes positioning a fluidic device at **554** on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. Furthermore, the method **550** may include moving at **556** the locator arm to the biased position. The locator arm can press the device against the reference surfaces such that the device is held in a fixed position.

FIGS. **28-37** illustrate various features of a fluid storage system **1000** (FIG. **28**). The storage system **1000** is configured to store and regulate a temperature of various fluids that may be used during predetermined assays. The storage system **1000** may be used by the workstation **160** (FIG. **2**) and enclosed by the casing **162** (FIG. **3**). As shown in FIG. **28**, the storage system **1000** includes an enclosure **1002** having a base shell (or first shell) **1004** and a top shell (or second shell) **1006** that are coupled together and define a system cavity **1008** therebetween. The enclosure **1002** may also include a system door **1010** that is configured to open and provide access to the system cavity **1008**. Also shown, the storage system **1000** may include a temperature-control assembly **1012** that is coupled to a rear of the enclosure **1002** and an elevator drive motor **1014** that is located on the top shell **1006**.

FIG. **29** is a side cross-section of the storage system **1000** and illustrates the system cavity **1008** in greater detail. The storage system **1000** may also include a reaction component tray (or reaction component storage unit) **1020** and a fluid removal assembly **1022** that includes an elevator mechanism **1024**. The tray **1020** is configured to hold a plurality of tubes or containers for storing fluids. The elevator mechanism **1024** includes the drive motor **1014** and is configured to move components of the removal assembly **1022** bi-directionally along the Z-axis. In FIG. **29**, the tray **1020** is located in a fluid-removal position such that fluid held by the tray **1020** may be removed and delivered to, for example, a fluidic device for performing a desired reaction or for flushing the flow channels of the fluidic device.

Also shown, the temperature-control assembly **1012** may project into the system cavity **1008**. The temperature-control assembly **1012** is configured to control or regulate a temperature within the system cavity **1008**. In the illustrated embodiment, the temperature-control assembly **1012** includes a thermo-electric cooling (TEC) assembly.

FIG. **30** is a perspective view of the removal assembly **1022**. As shown, the removal assembly **1022** may include a pair of opposing guide rails **1032** and **1034**. The opposing guide rails **1032** and **1034** are configured to receive and direct the tray **1020** to the fluid-removal position shown in FIG. **29**. The guide rails **1032** and **1034** may include projected features or ridges **1035** that extend longitudinally along the guide rails **1032** and **1034**. The guide rails **1032** and **1034** are configured to be secured to the base shell **1004** (FIG. **28**). The removal assembly **1022** also includes support beams (or uprights) **1036** and **1038** that extend in a direction along the Z-axis. A guide plate **1040** of the removal assembly may be coupled to the support beams **1036** and **1038** at an elevated distance  $D_z$  and project therefrom along the XY-plane. In the illustrated embodiment, the guide plate **1040** is affixed to the support beams **1036** and **1038**.

The elevator mechanism **1024** includes structural supports **1041** and **1042**, a lead screw **1044** that extends between the structural supports **1041** and **1042**, and a stage assembly **1046** that includes a transport platform **1048**. The structural supports **1041** and **1042** are secured to opposite ends of the support beams **1036** and **1038** and are configured to support the elevator mechanism **1024** during operation.



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Threads of the lead screw **1044** are operatively coupled to the stage assembly **1046** such that when the lead screw **1044** is rotated, the stage assembly **1046** moves in a linear direction along the Z-axis (indicated by the double arrows).

The transport platform **1048** is configured to hold an array of sipper tubes **1050**. The sipper tubes **1050** may be in fluid communication with a system pump (not shown) that is configured to direct a flow of fluid through the sipper tubes **1050**. As shown, the sipper tubes **1050** include distal portions **1052** that are configured to be inserted into component wells **1060** (shown in FIG. 31) of the tray **1020**. The distal portions **1052** extend through corresponding openings **1053** of the guide plate **1040**.

The elevator mechanism **1024** is configured to move the sipper tubes **1050** between withdrawn and deposited levels. At the deposited level (shown in FIGS. 50 and 51), the distal portions **1052** of the sipper tubes **1050** are inserted into the component wells **1060** to remove fluid therefrom. At the withdrawn level, the distal portions **1052** are completely removed from the tray **1020** such that the tray **1020** may be removed from the system cavity **1008** (FIG. 28) without damage to the sipper tubes **1050** or the tray **1020**. More specifically, when the drive motor **1014** rotates the lead screw **1044**, the stage assembly **1046** moves along the Z-axis in a direction that is determined by a rotational direction of the lead screw **1044**. Consequently, the transport platform **1048** moves along the Z-axis while holding the sipper tubes **1050**. If the transport platform **1048** advances toward the guide plate **1040**, the distal portions **1052** slide through the corresponding openings **1053** of the guide plate **1040** toward the tray **1020**. The guide plate **1040** is configured to prevent distal portions **1052** from becoming misaligned with the component wells **1060** before the distal portions **1052** are inserted therein. When the elevator mechanism **1024** moves the stage assembly **1046** away from the guide plate **1040**, a distance ( $\Delta Z$ ) between the transport platform **1048** and the guide plate **1040** increases until the distal portions **1052** are withdrawn from the component wells **1060** of the tray **1020**.

FIG. 30 illustrates additional features for operating the elevator mechanism **1024**. For example, the stage assembly **1046** may also include a guide pin **1058** (also shown in FIG. 29) that is affixed to and extends from the transport platform **1048** in a direction that is parallel to the sipper tubes **1050**. The guide pin **1058** also extends through a corresponding opening **1053** of the guide plate **1040**. In the illustrated embodiment, the guide pin **1058** extends a greater distance than the sipper tubes **1050** so that the guide pin **1058** reaches the tray **1020** before the sipper tubes **1050** are inserted into the component wells **1060**. Thus, if the tray **1020** is misaligned with respect to the sipper tubes **1050**, the guide pin **1058** may engage the tray **1020** and adjust the position of the tray **1020** so that the component wells **1060** are properly aligned with the corresponding sipper tubes **1050** before the sipper tubes **1050** are inserted therein.

In addition to the above, the removal assembly **1022** may include a position sensor **1062** and a location sensor (not shown). The position sensor **1062** is configured to receive a flag **1063** (shown in FIG. 34) of the tray **1020** to determine that the tray **1020** is present in the system cavity **1008** (FIG. 28) and at least approximately aligned for receiving the sipper tubes **1050**. The location sensor may detect a flag **1064** of the stage assembly **1046** to determine a level of the stage assembly **1046**. If the flag **1064** has not reached a threshold level along the Z-axis, the location sensor may communicate with the workstation **160** (or other assay system) to notify the user that the tray **1020** is not ready for

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removal. The workstation **160** could also prevent the user from opening the system door **1010**.

Furthermore, when the distal portions **1052** of the sipper tubes **1050** are initially inserted into the component wells **1060**, the sipper tubes **1050** may pierce protective foils that cover the component wells **1060**. In some instances, the foils may grip the sipper tubes **1050**. When the sipper tubes **1050** are subsequently withdrawn from the corresponding component wells **1060**, the gripping of the protective foils may collectively lift the tray **1020**. However, in the illustrated embodiment, the ridges **1035** are configured to grip a tray base **1070** (FIG. 31) and prevent the tray base **1070** from being lifted in a direction along the Z-axis. For example, the ridges **1035** may grip a lip **1071** of the tray base **1070**.

FIGS. 31-34 illustrate different views of the tray **1020**. The tray **1020** is configured to hold a plurality of component wells **1060**. The component wells **1060** may include various reaction components, such as, but not limited to, one or more samples, polymerases, primers, denaturants, linearization mixes for linearizing DNA, enzymes suitable for a particular assay (e.g., cluster amplification or SBS), nucleotides, cleavage mixes, oxidizing protectants, and other reagents. In some embodiments, the tray **1020** may hold all fluids that are necessary to perform a predetermined assay. In particular embodiments, the tray **1020** may hold all reaction components necessary for generating a sample (e.g., DNA clusters) within a flow cell and performing sample analysis (e.g., SBS). The assay may be performed without removing or replacing any of the component wells **1060**.

The component wells **1060** include rectangular component wells **1060A** (shown in FIGS. 35-36) and tubular component wells **1060B** (shown in FIG. 37). The tray **1020** includes a tray base **1070** and a tray cover **1072** coupled to the tray base **1070**. As shown in FIGS. 31 and 32, the tray cover **1072** includes a handle **1074** that is sized and shaped to be gripped by a user of the tray **1020**. The tray cover **1072** may also include a grip recess **1076** that is sized and shaped to receive one or more fingers of the user.

As shown in FIGS. 31 and 32, the tray cover **1072** may include a plurality of tube openings **1080** that are aligned with corresponding component wells **1060**. The tube openings **1080** may be shaped to direct the sipper tubes **1050** (exemplary sipper tubes **1050** are shown in FIG. 31) into the corresponding component wells **1060**. As shown in FIG. 32, the tray cover **1072** also includes a pin opening **1082** that is sized and shaped to receive the guide pin **1058**. The guide pin **1058** is configured to provide minor adjustments to the position of the tray **1020** if the guide pin **1058** approaches and enters the pin opening **1082** in a misaligned manner. Also shown, the tray **1020** may include an identification tag **1084** along a surface of the tray cover **1072**. The identification tag **1084** is configured to be detected by a reader to provide the user with information regarding the fluids held by the component wells **1060**.

As shown in FIGS. 33 and 34, the tube openings **1080** are at least partially defined by rims **1086** that project from a surface **1073** of the tray cover **1072**. The rims **1086** project a small distance away from the surface **1073** to prevent inadvertent mixing of fluids that are accidentally deposited onto the tray cover **1072**. Likewise, the identification tag **1084** may be attached to a raised portion **1088** of the tray cover **1072**. The raised portion **1088** may also protect the identification tag **1084** from inadvertently contacting fluids.

FIG. 35 shows a side cross-sectional view of the component well **1060A**, and FIG. 36 shows a bottom perspective view of the component well **1060A**. As shown, the component well **1060A** includes opposite first and second ends



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1091 and 1092 and a reservoir 1090 (FIG. 35) extending therebetween. The reservoir 1090 has a depth  $D_R$  (FIG. 35) that increases as the reservoir 1090 extends from the second end 1092 to the first end 1091. The component well 1060A is configured to receive the sipper tube 1050 in a deeper portion of the reservoir 1090. As shown in FIG. 36, the component well 1060A includes a plurality of projections 1094 along an exterior surface that are configured to rest upon a surface of the tray base 1070.

FIG. 37 is a perspective view of the component well 1060B. As shown, the component well 1060B may also include a plurality of projections 1096 around an exterior surface of the component well 1060B. The component well 1060B extends along a longitudinal axis 1097 and has a profile that tapers as the component well 1060B extends longitudinally to a bottom 1098. The bottom 1098 may have a substantially planar surface.

FIG. 61 illustrates a method 960 for performing an assay for biological or chemical analysis. In some embodiments, the assay may include a sample generation protocol and a sample analysis protocol. For example, the sample generation protocol may include generating clusters of DNA through bridge amplification and the sample analysis protocol may include sequencing-by-synthesis (SBS) analysis using the clusters of DNA. The sample generation and sample analysis operations may be conducted within a common assay system, such as the assay system 100 or the workstation 160, and without user intervention between the operations. For instance, a user may be able to load a fluidic device into the assay system. The assay system may automatically generate a sample for analysis and carry out the steps for performing the analysis.

With respect to FIG. 61, the method 960 includes establishing at 962 a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components. The reaction components may be configured for conducting one or more assays. The fluidic device may be, for example, the fluidic device 300 or the flow cell 200 described above. In some embodiments, the sample area includes a plurality of reaction components (e.g., primers) immobilized thereon. The storage unit may be, for example, the storage unit 1020 described above. The reaction components may include sample-generation components that are configured to be used to generate the sample, and sample-analysis components that are configured to be used to analyze the sample. In particular embodiments, the sample-generation components include reaction components for performing bridge amplification as described above. Furthermore, in particular embodiments, the sample-analysis components include reaction components for performing SBS analysis as described above.

The method 960 also includes generating at 964 a sample at the sample area of the fluidic device. The generating operation 964 may include flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. For example, a thermocycler may be used to facilitate hybridizing nucleic acids. However, isothermal methods can be used if desired. Furthermore, a flow rate of the fluids may be controlled to permit hybridization or other desired chemical reactions. In particular embodiments, the generating operation 964 includes conducting multiple bridge-amplification cycles to generate a cluster of DNA.

An exemplary protocol for bridge amplification can include the following steps. A flow cell is placed in fluid communication with a reaction component storage unit. The

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flow cell includes one or more surfaces to which are attached pairs of primers. A solution having a mixture of target nucleic acids of different sequences is contacted with a solid support. The target nucleic acids can have common priming sites that are complementary to the pairs of primers on the flow cell surface such that the target nucleic acids bind to a first primer of the pairs of primers on the flow cell surface. An extension solution containing polymerase and nucleotides can be introduced to the flow cell such that a first amplification product, which is complementary to the target nucleic acid, is formed by extension of the first primer. The extension solution can be removed and replaced with a denaturation solution. The denaturation solution can include chemical denaturants such as sodium hydroxide and/or formamide. The resulting denaturation conditions release the original strand of the target nucleic acid, which can then be removed from the flow cell by removing the denaturation solution and replacing it with the extension solution. In the presence of the extension solution the first amplification product, which is attached to the support, can then hybridize with a second primer of the primer pairs attached to the flow cell surface and a second amplification product comprising an attached nucleic acid sequence complementary to the first amplification product can be formed by extension of the second primer. Repeated delivery of the denaturation solution and extension solution can be used to form clusters of the target nucleic acid at discrete locations on the surface of the flow cell. Although the above protocol is exemplified using chemical denaturation, it will be understood that thermal denaturation can be carried out instead albeit with similar primers and target nucleic acids. Further description of amplification methods that can be used to produce clusters of immobilized nucleic acid molecules is provided, for example, in U.S. Pat. No. 7,115,400; U.S. Publication No. 2005/0100900; WO 00/18957; or WO 98/44151, each of which is incorporated by reference herein.

The method 960 also includes analyzing at 966 the sample at the sample area. Generally, the analyzing operation 966 may include detecting any detectable characteristic at the sample area. In particular embodiments, the analyzing operation 966 includes flowing at least one sample-analysis component to the sample area. The sample-analysis component may react with the sample to provide optically detectable signals that are indicative of an event-of-interest (or desired reaction). For example, the sample-analysis components may be fluorescently-labeled nucleotides used during SBS analysis. When excitation light is incident upon the sample having fluorescently-labeled nucleotides incorporated therein, the nucleotides may emit optical signals that are indicative of the type of nucleotide (A, G, C, or T), and the imaging system may detect the optical signals.

A particularly useful SBS protocol exploits modified nucleotides having removable 3' blocks, for example, as described in WO 04/018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. Repeated cycles of SBS reagents can be delivered to a flow cell having target nucleic acids attached thereto, for example, as a result of the bridge amplification protocol set forth above. The nucleic acid clusters can be converted to single stranded form using a linearization solution. The linearization solution can contain, for example, a restriction endonuclease capable of cleaving one strand of each cluster. Other methods of cleavage can be used as an alternative to restriction enzymes or nicking enzymes, including inter alia chemical cleavage (e.g., cleavage of a diol linkage with periodate), cleavage of abasic sites by cleavage with endonuclease (for example 'USER', as supplied by NEB, Ips-



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wich, MA, USA, part number M5505S), by exposure to heat or alkali, cleavage of ribonucleotides incorporated into amplification products otherwise comprised of deoxyribonucleotides, photochemical cleavage or cleavage of a peptide linker. After the linearization step a sequencing primer can be delivered to the flow cell under conditions for hybridization of the sequencing primer to the target nucleic acids that are to be sequenced.

The flow cell can then be contacted with an SBS extension reagent having modified nucleotides with removable 3' blocks and fluorescent labels under conditions to extend a primer hybridized to each target nucleic acid by a single nucleotide addition. Only a single nucleotide is added to each primer because once the modified nucleotide has been incorporated into the growing polynucleotide chain complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase cannot add further nucleotides. The SBS extension reagent can be removed and replaced with scan reagent containing components that protect the sample under excitation with radiation. Exemplary components for scan reagent are described in US publication US 2008/0280773 A1 and U.S. Ser. No. 13/018,255, each of which is incorporated herein by reference. The extended nucleic acids can then be fluorescently detected in the presence of scan reagent. Once the fluorescence has been detected, the 3' block may be removed using a deblock reagent that is appropriate to the blocking group used. Exemplary deblock reagents that are useful for respective blocking groups are described in WO04018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. The deblock reagent can be washed away leaving target nucleic acids hybridized to extended primers having 3' OH groups that are now competent for addition of a further nucleotide. Accordingly the cycles of adding extension reagent, scan reagent, and deblock reagent, with optional washes between one or more of the steps, can be repeated until a desired sequence is obtained. The above cycles can be carried out using a single extension reagent delivery step per cycle when each of the modified nucleotides has a different label attached thereto, known to correspond to the particular base. The different labels facilitate discrimination between the bases added during each incorporation step. Alternatively, each cycle can include separate steps of extension reagent delivery followed by separate steps of scan reagent delivery and detection, in which case two or more of the nucleotides can have the same label and can be distinguished based on the known order of delivery.

Continuing with the example of nucleic acid clusters in a flow cell, the nucleic acids can be further treated to obtain a second read from the opposite end in a method known as paired end sequencing. Methodology for paired end sequencing are described in PCT publication WO07010252, PCT application Serial No. PCTGB2007/003798 and US patent application publication US 2009/0088327, each of which is incorporated by reference herein. In one example, a series of steps may be performed as follows; generate clusters as set forth above, linearize as set forth above, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above, "invert" the target nucleic acids on the flow cell surface by synthesizing a complementary copy, linearize the resynthesized strand, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above. The inversion step can

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be carried out by delivering reagents as set forth above for a single cycle of bridge amplification.

Although the analyzing operation has been exemplified above with respect to a particular SBS protocol, it will be understood that other protocols for sequencing any of a variety of other molecular analyses can be carried out as desired. Appropriate modification of the apparatus and methods to accommodate various analyses will be apparent in view of the teaching set forth herein and that which is known about the particular analysis method.

In some embodiments, the method 960 is configured to be conducted with minimal user intervention. The generating and analyzing operations 964 and 966 may be conducted in an automated manner by an assay system. For example, in some cases, a user may only load the fluidic device and the storage unit and activate the assay system to perform the method 960. In some embodiments, during the generating and analyzing operations 964 and 966, the storage unit and the fluidic device remain in fluid communication from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. In other words, the fluidic device and the storage unit may remain in fluid communication from before the sample is generated until after the sample is analyzed. In some embodiments, the fluidic device is continuously held by the device holder from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. During such time, the device holder and an imaging lens may be automatically moved with respect to each other. The storage unit and the fluidic device may remain in fluid communication when the fluidic device and the imaging lens are automatically moved with respect to each other. In some embodiments, the assay system is contained within a workstation housing and the generating and analyzing operations 964 and 966 are conducted exclusively within the workstation housing.

FIG. 38 is a schematic illustration of an optical imaging system 600 formed in accordance with one embodiment. The imaging system 600 includes an optical assembly 602, a light source (or excitation light) module or assembly 604, a flow cell 606 having a sample area 608, and imaging detectors 610 and 612. The light source module 604 includes first and second excitation light sources 614 and 616 that are configured to illuminate the sample area 608 with different excitation spectra. In particular embodiments, the first and second excitation light sources 614 and 616 comprise first and second semiconductor light sources (SLSs). SLSs may include light-emitting diodes (LEDs) or laser diodes. However, other light sources may be used in other embodiments, such as lasers or arc lamps. The first and second SLSs may have fixed positions with respect to the optical assembly 602.

As shown, the optical assembly 602 may include a plurality of optical components. For example, the optical assembly 602 may include lenses 621-627, emission filters 631-634, excitation filters 635 and 636, and mirrors 641-645. The plurality of optical components are arranged to at least one of (a) direct the excitation light toward the sample area 608 of the flow cell 606 or (b) collect emission light from the sample area 608. Also shown, the imaging system 600 may also include a flow system 652 that is in fluid communication with the flow cell 606 and a system controller 654 that is communicatively coupled to the first and second excitation light sources 614 and 616 and the flow system 652. The controller 654 is configured to activate the



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flow system **652** to flow reagents to the sample area **608** and activate the first and second SLSSs after a predetermined time period.

For example, FIG. **60** illustrates a method **900** for performing an assay for biological or chemical analysis. In particular embodiments, the assay may include a sequencing-by-synthesis (SBS) protocol. The method **900** includes flowing reagents through a flow channel of a flow cell at **902**. The flow cell may have a sample area that includes a sample with biomolecules configured to chemically react with the reagents. The method **900** also includes illuminating the sample area at **904** with first and second semiconductor light sources (SLSSs). The first and second SLSSs provide first and second excitation spectra, respectively. The biomolecules of the sample may provide light emissions that are indicative of a binding reaction when illuminated by the first or second SLSSs. Furthermore, the method **900** includes detecting the light emissions from the sample area at **906**. Optionally, the method **900** may include moving the flow cell at **908** relative to an imaging lens and repeating the illuminating and detecting operations **904** and **906**. The steps shown in FIG. **60** and exemplified above can be repeated for multiple cycles of a sequencing method.

FIGS. **39** and **40** illustrate various features of a motion-control system **700** formed in accordance with one embodiment that may be used with the imaging system **600**. The motion-control system **700** includes an optical base plate **702** and a sample deck **708** that is movably coupled to the base plate **702**. As shown, the base plate **702** has a support side **704** and a bottom side **705**. The support and bottom sides **704** and **705** face in opposite directions along the Z-axis. The base plate **702** is configured to support a majority of the optical components of the optical assembly **602** (FIG. **38**) on the support side **704**. The base plate **702** and the sample deck **708** may be movably coupled to each other by an intermediate support **715** and a face plate **722** such that the sample holder **650** may substantially rotate about the X and Y axes, shift along the Y axis, and slide along the X axis.

FIG. **40** is an isolated perspective view of the intermediate support **715**, a motor assembly **724**, and a movable platform **726** of the sample deck **708** (FIG. **39**). The motor assembly **724** is operatively coupled to the platform **726** and is configured to slide the platform **726** bi-directionally along the X-axis. As shown, the intermediate support **715** includes a tail end **728** and an imaging end **730**. The intermediate support **715** may include pins **746** and **748** proximate to the imaging end **730** that project away from each other along the Y-axis. Proximate to the imaging end **730**, the intermediate support **715** may include a lens opening **750** that is sized and shaped to allow the imaging lens **623** (FIG. **38**) to extend therethrough. In the illustrated embodiment, the pins **746** and **748** have a common line **755** extending therethrough that also extends through the lens opening **750**.

Returning to FIG. **39**, the platform **726** is coupled to the bottom side **705** through the intermediate support **715**. Accordingly, a weight of the sample deck **708** may be supported by the base plate **702**. Furthermore, the motion-control system **700** may include a plurality of alignment devices **733**, **735**, **737**, and **739** that are configured to position the sample holder **650**. In the illustrated embodiment, the alignment devices **733**, **735**, **737**, and **739** are micrometers. The alignment device **733** is operatively coupled to the tail end **728** of the intermediate support **715**. When the alignment device **733** is activated, the tail end **728** may be moved in a direction along the Z-axis. Consequently, the intermediate support **715** may rotate about the pins **746**

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and **748** (FIG. **40**) or, more specifically, about the line **755**. When the alignment devices **735** and **737** are activated, the sample holder **650** may shift along the Y-axis as directed. When the alignment device **739** is activated, the sample holder **650** may rotate about an axis of rotation  $R_7$  that extends parallel to the X-axis.

FIGS. **41-42** show a perspective view and plan view, respectively, of the optical base plate **702** that may be used with the imaging system **600** (FIG. **38**). In some embodiments of the imaging system **600**, one or more of the optical components **621-627**, **631-636**, and **641-645** (FIG. **38**) can have a fixed position in the optical assembly **602** such that the fixed (or static) optical component does not move during operation of the imaging system **600**. For example, the base plate **702** is configured to support a plurality of optical components and other parts of the imaging system **600**. As shown, the base plate **702** constitutes a substantially unitary structure having a support side (or surface) **704** that faces in a direction along the Z-axis. In the illustrated embodiment, the support side **704** is not continuously smooth, but may have various platforms **716-718**, depressions (or receiving spaces) **719-721**, and component-receiving spaces **711-714** that are located to arrange the optical assembly **602** in a predetermined configuration. As shown in FIG. **42**, each of the component-receiving spaces **711-714** has respective reference surfaces **781-784**. In some embodiments, the reference surfaces **781-784** can facilitate orienting and holding corresponding optical components in desired positions.

FIGS. **43** and **44** show a front perspective view and a cutaway rear perspective view, respectively, of an optical device **732**. As shown in FIG. **43**, the optical device **732** is oriented relative to mutually perpendicular axes **791-793**. The axis **791** may extend along a gravitational force direction and/or parallel to the Z-axis illustrated above. In particular embodiments, the optical device **732** is configured to be positioned within the component-receiving space **713** (FIG. **43**) of the base plate **702** (only a portion of the base plate **702** is shown in FIGS. **43** and **44**).

The component-receiving space **713** has one or more surfaces that define an accessible spatial region where an optical component may be held. These one or more surfaces may include the reference surface(s) described below. In the illustrated embodiment, the component-receiving space **713** is a component cavity of the base plate **701** that extends a depth within the base plate **702**. However, the base plate **702** may form the component-receiving space in other manners. For example, in a similar way that the base plate **702** may form a cavity, the base plate **702** may also have one or more raised platforms including surfaces that surround and define the component-receiving space. Accordingly, the base plate **702** may be shaped to partially or exclusively provide the component-receiving space. The base plate **702** may include the reference surface. In alternative embodiments, sidewalls may be mounted on the base plate **702** and configured to define the spatial region. Furthermore, other optical devices mounted to the base plate **702** may define the component-receiving spaces. As used herein, when an element "defines" a component-receiving space, the element may exclusively define the component-receiving space or may only partially define the component-receiving space.

The optical device **732** can be removably mounted to the base plate **702** in the component-receiving space **713**, but may be configured to remain in a fixed position during operation of the imaging system. However, in alternative embodiment, the optical device **732** is not positioned within the component-receiving space **713**, but may be positioned elsewhere, such as on a platform of the support side **704**. In



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the illustrated embodiment, the optical device **732** includes a mounting device **734** and an optical component **736** that is configured to reflect and/or transmit light therethrough. The mounting device **734** is configured to facilitate holding the optical component **736** in a desired orientation and also removably mount the optical component **736** to the base plate **702**. The mounting device **734** includes a component retainer **738** and a biasing element **740** that is operatively coupled to the retainer **738**.

In the illustrated embodiment, the optical component **736** comprises an optical filter that transmits optical signals therethrough while filtering for a predetermined spectrum. However, other optical components may be used in alternative embodiments, such as lenses or mirrors. As shown, the optical component **736** may include optical surfaces **742** and **744** that face in opposite directions and define a thickness  $T_3$  of the optical component **736** therebetween. As shown, the optical surfaces **742** and **744** may be continuously smooth and planar surfaces that extend parallel to each other such that the thickness  $T_3$  is substantially uniform. However, the optical surfaces **742** and **744** may have other contours in alternative embodiments. The optical component **736** may have a plurality of component edges **751-754** (FIG. **43**) that define a perimeter or periphery. The periphery surrounds the optical surfaces **742** and **744**. As shown, the periphery is substantially rectangular, but other geometries may be used in alternative embodiments (e.g., circular).

The retainer **738** facilitates holding the optical component **736** in a desired orientation. In the illustrated embodiment, the retainer **738** is configured to engage the optical surface **742** and extend around at least a portion of the periphery to retain the optical component **736**. For example, the retainer **738** may include a wall portion **756** and a frame extension **758** that extends from the wall portion **756** along the periphery of the optical component **736** (e.g., the component edge **752** (FIG. **43**)). In the illustrated embodiment, the frame extension **758** may form a bracket that limits movement of the optical component **736**. More specifically, the frame extension **758** may include a proximal arm **760** and a distal arm **762**. The proximal arm **760** extends from the wall portion **756** along the component edge **752** and the axis **791**. The distal arm **762** extends from the proximal arm **760** along the component edge **751**. The distal arm **762** includes a projection or feature **764** that extends toward and engages the optical component **736**. Also shown, the retainer **738** may include a grip member **766** that is located opposite the frame extension **758**. The grip member **766** and the frame extension **758** may cooperate in limiting movement of the optical component **736** along the axis **793**. The retainer **738** may grip a portion of the periphery of the optical component **736**.

As shown in FIGS. **43** and **44**, the wall portion **756** is configured to engage the optical surface **742**. For example, the wall portion **756** has a mating surface **770** (FIG. **43**) that faces the optical component **736**. In some embodiments, the wall portion **756** includes a plurality of orientation features **771-773** (FIG. **43**) along the mating surface **770**. The orientation features **771-773** are configured to directly engage the optical surface **742** of the optical component **736**. When the orientation features **771-773** directly engage the optical surface **742**, the optical surface **742** (and consequently the optical component **736**) is positioned in a desired orientation with respect to the retainer **738**. As shown in FIG. **43**, the reference surface **783** of the component-receiving space **713** also includes a plurality of orientation features **761-763**. The orientation features **761-763** are configured to directly engage the optical surface **744**. Further-

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more, the orientation features **761-763** may be arranged such that each of the orientation features **761-763** generally opposes a corresponding one of the orientation features **771-773**.

Also shown in FIG. **44**, the wall portion **756** has a non-mating surface **774** that faces in an opposite direction with respect to the mating surface **770** (FIG. **43**). The wall portion **756** includes an element projection **776** that extends away from the non-mating surface **774** and the optical component **736**. The biasing element **740** is configured to couple to the element projection **776**. In the illustrated embodiment, the element projection **776** and the biasing element **740** extend into a slot **778** of the component-receiving space **713**. The slot **778** is sized and shaped to receive the biasing element **740**. The slot **778** has an element surface **780** that engages the biasing element **740**.

FIG. **45** shows an isolated front view of the optical device **732**, and FIG. **46** shows how the optical device **732** may be removably mounted to the base plate **702**. To removably mount the optical component **736**, the optical component **736** may be positioned within a component-receiving space **789** of the mounting device **734** that is generally defined by the wall portion **756** (FIG. **46**), the frame extension **758**, and the grip member **766**. In particular embodiments, when the optical component **736** is positioned within the mounting device **734**, the optical component **736** is freely held within the component-receiving space **789**. For instance, the optical component **736** may not form an interference fit with the retainer **738**. Instead, during a mounting operation, the optical component **736** may be held within the component-receiving space **789** by the wall portion **756**, the frame extension **758**, the grip member **766** and, for example, an individual's hand. However, in alternative embodiments, the optical component **736** may form an interference fit with the retainer **738** or may be confined within a space that is defined only by the retainer **738**.

With respect to FIG. **46**, during the mounting operation, the biasing element **740** may be initially compressed so that the mounting device **734** may clear and be inserted into the component-receiving space **713**. For example, the biasing element **740** may be compressed by an individual's finger to reduce the size of the optical device **732**, or the biasing element **740** may be compressed by first pressing the biasing element **740** against the element surface **780** and then advancing the retainer **738** into the component-receiving space **713**. Once the optical device **732** is placed within the component-receiving space **713**, the stored mechanical energy of the compressed biasing element **740** may move the retainer **738** and the optical component **736** toward the reference surface **783** until the optical surface **744** directly engages the reference surface **783**. More specifically, the optical surface **744** may directly engage the orientation features **761-763** (FIG. **43**) of the reference surface **783**. As shown in FIG. **46**, when the optical component **736** is mounted, a small gap  $G_1$  may exist between the optical surface **742** and the mating surface **770** (FIG. **43**) because of the orientation features **771-773** (FIG. **43**), and a small gap  $G_2$  may exist between the optical surface **744** and the reference surface **783** because of the orientation features **761-763** (FIG. **43**).

In the mounted position, the biasing element **740** provides an alignment force  $F_A$  that holds the optical surface **744** against the reference surface **783**. The optical and reference surfaces **744** and **783** may be configured to position the optical component **736** in a predetermined orientation. The alignment force  $F_A$  is sufficient to hold the optical component **736** in the predetermined orientation throughout opera-



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tion of the imaging system. In other words, the mounting device **734** and the reference surface **783** may prevent the optical component **736** from moving in a direction along the axis **792**. Furthermore, in the mounted position, the projection **764** (FIG. **43**) may press against the component edge **751** (FIG. **43**) to prevent the optical component **736** from moving in a direction along the axis **791**. The frame extension **758** and the grip member **766** may prevent or limit movement of the optical component **736** in a direction along the axis **793**. Accordingly, the component-receiving space **713** and the mounting device **734** may be configured with respect to each other to hold the optical component **736** in a predetermined orientation during imaging sessions.

As shown in FIG. **45**, when the optical component **736** is in the mounted position, a space portion **798** of the optical surface **744** may face and interface with the reference surface **783**, and a path portion **799** of the optical surface **744** may extend beyond the support side **704** into an optical path taken by optical signals. Also shown in FIG. **46**, the component-receiving space **713** may extend a depth  $D_C$  into the base plate **702** from the support side **704**.

The biasing element **740** may comprise any elastic member capable of storing mechanical energy to provide the alignment force  $F_A$ . In the illustrated embodiment, the elastic member comprises a coil spring that pushes the optical surface **744** against the reference surface **783** when compressed. However, in alternative embodiments, the elastic member and the component-receiving space may be configured such that the elastic member pulls the optical surface against the reference surface when extended. For example, a coil spring may have opposite ends in which one end is attached to the element surface in a slot that extends from the reference surface and another end is attached to the retainer. When the coil spring is extended, the coil spring may provide an alignment force that pulls the optical component against the reference surface. In this alternative embodiment, a rubber band may also be used.

In alternative embodiments, the mounting device **734** may be used to affix the optical component **736** to the base plate **702** using an adhesive. More specifically, the optical component **736** may be held against the reference surface **783** by the mounting device **734**. An adhesive may be deposited into the gap  $G_2$  between the optical surface **744** and the reference surface **783**. After the adhesive cures, the mounting device **734** may be removed while the optical component **736** remains affixed to the reference surface **783** by the adhesive.

FIG. **47** is a block diagram illustrating a method **800** of assembling an optical train. The method **800** includes providing an optical base plate at **802** that has a component-receiving space. The base plate and the component-receiving space may be similar to the base plate **702** and the component-receiving space **713** described above. The method **800** also includes inserting an optical component at **804** into the component-receiving space. The optical component may be similar to the optical component **736** described above and include an optical surface that is configured to reflect or transmit light therethrough. The optical surface may have a space portion that faces a reference surface of the component-receiving space and a path portion that extends beyond the support side into an optical path. The method **800** also includes providing an alignment force at **806** that holds the optical surface against the reference surface to orient the optical component. The optical and reference surfaces may be configured to hold the optical component in a predetermined orientation when the alignment force is provided. In some embodiments, the method **800** may also include removing the optical compo-

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nent at **808** and, optionally, inserting a different optical component at **810** into the component-receiving space. The different optical component may have the same or different optical qualities. In other words, the different optical component may be a replacement that has the same optical qualities or the different optical component may have different optical qualities.

FIGS. **48** and **49** provide a perspective view and a side view, respectively, of the light source (or excitation light module) **604**. As used herein, a light source module includes one or more light sources (e.g., lasers, arc lamps, LEDs, laser diodes) that are secured to a module frame and also includes one or more optical components (e.g., lenses or filters) that are secured to the module frame in a fixed and predetermined position with respect to said one or more light sources. The light source modules may be configured to be removably coupled within an imaging system so that a user may relatively quickly install or replace the light source module. In particular embodiments, the light source module **604** constitutes a SLS module **604** that includes the first and second SLSs **614** and **616**. As shown, the SLS module **604** includes a module frame **660** and a module cover **662**. A plurality of imaging components may be secured to the module frame **660** in fixed positions with respect to each other. For example, the first and second SLSs **614** and **616**, the excitation filter **635**, and the lenses **624** and **625** may be mounted onto the module frame **660**. In addition, the SLS module **604** may include first and second heat sinks **664** (FIG. **48**) and **666** that are configured to transfer thermal energy from the first and second SLSs **614** and **616**, respectively.

The SLS module **604** and the module frame **660** may be sized and shaped such that an individual could hold the SLS module **604** with the individual's hands and readily manipulate for installing into the imaging system **600**. As such, the SLS module **604** has a weight that an adult individual could support.

The SLS module **604** is configured to be placed within the module-receiving space **719** (FIG. **41**) and removably coupled to the base plate **702** (FIG. **41**). As shown, the module frame **660** has a plurality of sides including a mounting side **670** and an engagement face **671** (FIG. **48**). In the illustrated embodiment, the module frame **660** is substantially rectangular or block-shaped, but the module frame **660** may have other shapes in alternative embodiments. The mounting side **670** is configured to be mounted to the base plate **702** within the module-receiving space **719**. As such, at least a portion of the module-receiving space **719** may be shaped to receive and hold the SLS module **604**. Similar to the component-receiving space **713**, the module-receiving space **719** may be defined by one or more surfaces that provide an accessible spatial region where the SLS module **604** may be held. The surface(s) may be of the base plate **702**. For example, in the illustrated embodiment, the module-receiving space **719** is a depression of the base plate **702**. The mounting side **670** may have a contour that substantially complements the base plate **702** and, more specifically, the module-receiving space **719**. For example, the mounting side **670** may be substantially planar and include a guidance pin **672** (FIG. **49**) projecting therefrom that is configured to be inserted into a corresponding hole (not shown) in the base plate **702**. The guidance pin **672** may be a fastener (e.g., screw) configured to facilitate removably coupling the module frame **660** to the base plate **702**. In particular embodiments, the guidance pin **672** is inserted into the base plate **702** at a non-orthogonal angle. As shown in FIG. **49**, the heat sink **666** may be coupled to the module



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frame **660** such that an offset **676** exists from the mounting side **670** to the heat sink **666**.

The module frame **660** may include first and second light passages **682** and **684** that intersect each other at a passage intersection **685**. The SLSs **614** and **616** may be secured to the module frame **660** and have fixed positions with respect to each other. The SLSs **614** and **616** are oriented such that optical signals are substantially directed along optical paths through the respective light passages **682** and **684** toward the passage intersection **685**. The optical paths may be directed toward the excitation filter **635**. In the illustrated embodiment, the optical paths are perpendicular to one another until reaching the excitation filter **635**. The excitation filter **635** is oriented to reflect at least a portion of the optical signals generated by the SLS **616** and transmit at least a portion of the optical signals generated by the SLS **614**. As shown, the optical signals from each of the SLSs **614** and **616** are directed along a common path and exit the SLS module **604** through a common module window **674**. The module window **674** extends through the engagement face **671**.

FIG. **50** is a plan view of the SLS module **604** mounted onto the base plate **702**. In the illustrated embodiment, the SLS module **604** is configured to rest on the base plate **702** such that the gravitational force  $g$  facilitates holding the SLS module **604** thereon. As such, the SLS module **604** may provide an integrated device that is readily removed or separated from the optical assembly **600**. For example, after removing a housing (not shown) of the assay system or after receiving access to the optical assembly, the SLS module **604** may be grabbed by an individual and removed or replaced. When the SLS module **604** is located on the base plate **702**, the engagement face **671** may engage an optical device **680**. The optical device **680** may be adjacent to the module window **674** such that the optical signals generated by the SLS module **604** are transmitted through the optical device **680**.

Although the illustrated embodiment is described as using an SLS module with first and second SLSs, excitation light may be directed onto the sample in other manners. For example, the SLS module **604** may include only one SLS and another optical component (e.g., lens or filter) having fixed positions with respect to each other in a module frame. Likewise, more than two SLSs may be used. In a similar manner, light modules may include only one laser or more than two lasers.

However, embodiments described herein are not limited to only having modular excitation systems, such as the SLS module **604**. For example, the imaging system **600** may use a light source that is not mounted to a module frame. More specifically, a laser could be directly mounted to the base plate or other portion of the imaging system or may be mounted to a frame that, in turn, is mounted within the imaging system.

Returning to FIG. **38**, the imaging system **600** may have an image-focusing system **840** that includes the object or sample holder **650**, an optical train **842**, and the imaging detector **610**. The optical train **842** is configured to direct optical signals from the sample holder **650** (e.g., light emissions from the sample area **608** of the flow cell **606**) to a detector surface **844** of the imaging detector **610**. As shown in FIG. **38**, the optical train **842** includes the optical components **623**, **644**, **634**, **633**, **621**, **631**, and **642**. The optical train **842** may include other optical components. In the illustrated configuration, the optical train **842** has an object or sample plane **846** located proximate to the sample holder **650** and an image plane **848** located proximate to the

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detector surface **844**. The imaging detector **610** is configured to obtain object or sample images at the detector surface **844**.

In some embodiments, the image-focusing system **840** is configured to move the image plane **848** relative to the detector **610** and capture a test image. More specifically, the image plane **848** may be moved such that the image plane **848** extends in a non-parallel manner with respect to the detector surface **844** and intersects the detector surface **844**. A location of the intersection may be determined by analyzing the test image. The location may then be used to determine a degree-of-focus of the imaging system **600**. In particular embodiments, the image-focusing system **840** utilizes a rotatable mirror that is operatively coupled to an actuator for moving the rotatable mirror. However, the image-focusing system **840** may move other optical components that direct the optical signals to the detector surface **844**, or the image-focusing system **840** may move the detector **610**. In either case, the image plane **848** may be relatively moved with respect to the detector surface **844**. For example, the image-focusing system **840** may move a lens.

In particular embodiments, the imaging detector **610** is configured to obtain test images using a rotatable mirror **642** to determine a degree-of-focus of the imaging system **600**. As a result of the determined degree-of-focus, the imaging system **600** may move the sample holder **650** so that the object or sample is located within the sample plane **846**. For example, the sample holder **650** may be configured to move the sample area **608** in a  $z$ -direction a predetermined distance (as indicated by  $\Delta z$ ).

FIG. **51** is a plan view that illustrates several of the components in the image-focusing system **840**. As shown, the image-focusing system **840** includes a rotatable mirror assembly **850** that includes the mirror **642**, a mounting assembly **852** having the mirror **642** mounted thereon, and an actuator or rotation mechanism **854** that is configured to rotate the mounting assembly **852** and the mirror **642** about an axis of rotation  $R_6$ . The mirror **642** is configured to reflect optical signals **863** that are received from the sample area **608** (FIG. **38**) toward the imaging detector **610** and onto the detector surface **844**. In the illustrated embodiment, the mirror **642** reflects the optical signals **863** directly onto the detector surface **844** (i.e., there are no intervening optical components that redirect the optical signals **863**). However, in alternative embodiments, there may be additional optical components that affect the propagation of the optical signals **863**.

In the illustrated embodiment, the image-focusing system **840** also includes positive stops **860** and **862** that are configured to prevent the mirror **642** from rotating beyond predetermined rotational positions. The positive stops **860** and **862** have fixed positions with respect to the axis  $R_6$ . The mounting assembly **852** is configured to pivot about the axis  $R_6$  between the positive stops **860** and **862** depending upon whether sample images or test images are being obtained. Accordingly, the mirror **642** may be rotated between a test position (or orientation) and an imaging position (or orientation). By way of example only, the mirror **642** may be rotated from approximately  $5^\circ$  to approximately  $12^\circ$  about the axis  $R_6$  between the different rotational positions. In particular embodiments, the mirror **642** may be rotated approximately  $8^\circ$  about the axis  $R_6$ .

FIG. **52** is a perspective view of the mirror assembly **850**. As shown, the mounting assembly **852** includes an interior frame **864** and a support bracket **866**. The interior frame **864** is configured to couple to the mirror **642** and also to the



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support bracket **866**. The interior frame **864** and the support bracket **866** may interact with each other and a plurality of set screws **868** to provide minor adjustments to the orientation of the mirror **642**. As such, the mounting assembly **852** may constitute a gimbal mirror mount assembly. Also shown, the mounting assembly **852** is coupled to the rotation mechanism **854**. In the illustrated embodiment, the rotation mechanism **854** comprises a direct drive motor. However, a variety of alternative rotation mechanisms may be used, such as direct current (DC) motors, solenoid drivers, linear actuators, piezoelectric motors, and the like. Also shown in FIG. **52**, the positive stop **860** may have a fixed position with respect to the rotation mechanism **854** and the axis  $R_6$ .

As discussed above, the rotation mechanism **854** is configured to rotate or pivot the mirror **642** about the axis  $R_6$ . As shown in FIG. **52**, the mirror **642** has a geometric center C that extends along the axis  $R_6$ . The geometric center C of the mirror **642** is offset with respect to the axis  $R_6$ . In some embodiments, the rotation mechanism **854** is configured to move the mirror **642** between the test position and imaging position in less than 500 milliseconds. In particular embodiments, the rotation mechanism **854** is configured to move the mirror **642** between the test position and imaging position in less than 250 milliseconds or less than 160 milliseconds.

FIG. **53** is a schematic diagram of the mirror **642** in the imaging position. As shown, the optical signals **863** from the sample area **608** (FIG. **38**) are reflected by the mirror **642** and directed toward the detector surface **844** of the imaging detector **610**. Depending upon the configuration of the optical train **842** and the z-position of the sample holder **610**, the sample area **608** may be sufficiently in-focus or not sufficiently in-focus (i.e., out-of-focus). FIG. **53** illustrates two image planes **848A** and **848B**. The image plane **848A** substantially coincides with the detector surface **844** and, as such, the corresponding sample image has an acceptable or sufficient degree-of-focus. However, the image plane **848B** is spaced apart from the detector surface **844**. Accordingly, the sample image obtained when the image plane **848B** is spaced apart from the detector surface **844** may not have a sufficient degree-of-focus.

FIGS. **54** and **55** illustrate sample images **870** and **872**, respectively. The sample image **870** is the image detected by the imaging detector **610** when the image plane **848A** coincides with the detector surface **844**. The sample image **872** is the image detected by the imaging detector **610** when the image plane **848B** does not coincide with the detector surface **844**. (The sample images **870** and **872** include clusters of DNA that provide fluorescent light emissions when excited by predetermined excitation spectra.) As shown in FIGS. **54** and **55**, the sample image **870** has an acceptable degree-of-focus in which each of the clusters along the sample image **870** is clearly defined, and the sample image **872** does not have an acceptable degree-of-focus in which each of the clusters is clearly defined.

FIG. **56** is a schematic diagram of the mirror **642** in the focusing position. As shown, the mirror **642** in the focusing position has been rotated about the axis  $R_6$  an angle  $\theta$ . Again, the optical signals **863** from the sample area **608** (FIG. **38**) are reflected by the mirror **642** and directed toward the detector surface **844** of the imaging detector **610**. However, the optical train **842** in FIG. **56** is arranged so that the image plane **848** has been moved with respect to the detector surface **844**. More specifically, the image plane **848** does not extend parallel to the detector surface **844** and, instead, intersects the detector surface **844** at a plane intersection PI. While the mirror **642** is in the focusing position, the imaging system **600** may obtain a test image of the sample area **608**.

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As shown in FIG. **56**, the plane intersections PI may occur at different locations on the detector surface **844** depending upon the degree to which the sample area **608** is in-focus during an imaging session.

For example, FIGS. **57** and **58** illustrate test images **874** and **876**, respectively. The test image **874** represents the image obtained when the sample area **608** is in-focus, and the test image **876** represents the image obtained when the optical train **842** is out-of-focus. As shown, the test image **874** has a focused region or location  $FL_1$  that is located a distance  $XD_1$  away from a reference edge **880**, and the test image **876** has a focused region or location  $FL_2$  that is located a distance  $XD_2$  away from a reference edge **880**. The focused locations  $FL_1$  and  $FL_2$  may be determined by an image analysis module **656** (FIG. **38**).

To identify the focused locations  $FL_1$  and  $FL_2$  in the test images **874** and **876**, the image analysis module **656** may determine the location of an optimal degree-of-focus in the corresponding test image. More specifically, the analysis module **656** may determine a focus score for different points along the x-dimension of the test images **874** and **876**. The analysis module **656** may calculate the focus score at each point based on one or more image quality parameters. Examples of image quality parameters include image contrast, spot size, image signal to noise ratio, and the mean-square-error between pixels within the image. By way of example, when calculating a focus score, the analysis module **656** may calculate a coefficient of variation in contrast within the image. The coefficient of variation in contrast represents an amount of variation between intensities of the pixels in an image or a select portion of an image. As a further example, when calculating a focus score, the analysis module **656** may calculate the size of a spot derived from the image. The spot can be represented as a Gaussian spot and size can be measured as the full width half maximum (FWHM), in which case smaller spot size is typically correlated with improved focus.

After determining the focused location FL in the test image, the analysis module **656** may then measure or determine the distance XD that the focused location FL is spaced apart or separated from the reference edge **880**. The distance XD may then be correlated to a z-position of the sample area **608** with respect to the sample plane **846**. For example, the analysis module **656** may determine that the distance  $XD_2$  shown in FIG. **58** corresponds to the sample area **608** be located a distance  $\Delta z$  from the sample plane **846**. As such, the sample holder **650** may then be moved the distance  $\Delta z$  to move the sample area **608** within the sample plane **846**. Accordingly, the focused locations FL in test images may be indicative of a position of the sample area **608** with respect to the sample plane **846**. As used herein, the phrase “being indicative of a position of the object (or sample) with respect to the object (or sample) plane” includes using the factor (e.g., the focused location) to provide a more suitable model or algorithm for determining the distance  $\Delta z$ .

FIG. **59** is a block diagram illustrating a method **890** for controlling focus of an optical imaging system. The method **890** includes providing an optical train at **892** having a rotatable mirror that is configured to direct optical signals onto a detector surface. The detector surface may be similar to the detector surface **844**. The optical train may have an object plane, such as the sample plane **846**, that is proximate to an object. The optical train may also have an image plane, such as the image plane **848**, that is proximate to the detector surface. The rotatable mirror may be rotatable between an imaging position and a focusing position.



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The method **890** also includes rotating the mirror at **894** to the focusing position and obtaining a test image of the object at **896** when the mirror is in the focusing position. The test image may have an optimal degree-of-focus at a focused location. The focused location may be indicative of a position of the object with respect to the object plane. Furthermore, the method **890** may also include moving the object at **898** toward the object plane based on the focused location.

It is to be understood that the above description is intended to be illustrative, and not restrictive. For example, the above-described embodiments (and/or aspects thereof) may be used in combination with each other. In addition, many modifications may be made to embodiments without departing from the scope of the invention in order to adapt a particular situation or material. While the specific components and processes described herein are intended to define the parameters of the various embodiments, they are by no means limiting and are exemplary embodiments. Many other embodiments will be apparent to those of skill in the art upon reviewing the above description. The scope of the invention should, therefore, be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. In the appended claims, the terms “including” and “in which” are used as the plain-English equivalents of the respective terms “comprising” and “wherein.” Moreover, in the following claims, the terms “first,” “second,” and “third,” etc. are used merely as labels, and are not intended to impose numerical requirements on their objects. Further, the limitations of the following claims are not written in means-plus-function format and are not intended to be interpreted based on 35 U.S.C. § 112, sixth paragraph, unless and until such claim limitations expressly use the phrase “means for” followed by a statement of function void of further structure.

What is claimed is:

1. A system comprising:

- an optical system comprising an excitation light source, an imaging detector, and an optical train;
- a device holder to orient a fluidic device for imaging by the optical system, the fluidic device comprising a flow cell, the device holder comprising:
  - a support structure including a loading region to receive the fluidic device, the loading region including a base surface to have the fluidic device positioned thereon, and
  - a rotatable cover that is coupled to the support structure and moveable about an axis between an open position and a closed position, the cover moveable to the open position to permit the fluidic device to be inserted into and removed from the loading region and moveable to the closed position to secure the fluidic device within the loading region for imaging, wherein the cover is biased toward the open position, and
  - a latch to releasably hold the cover in the closed position;
- a fluid storage system comprising:
  - an enclosure having a cavity,
  - a door openable to provide access to the cavity,
  - a transport platform holding an array of sipper tubes, each sipper tube of the array of sipper tubes includes a distal portion positioned to be inserted into a component well of a reaction component tray within the cavity, and
  - a drive motor operatively coupled to the transport platform, the transport platform moveable by the

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drive motor to position the array of sipper tubes at least partially within the cavity; and

a casing enclosing the optical system, the device holder, and the fluid storage system therein.

2. The system of claim 1, wherein the cover comprises a compression arm to press the fluidic device against the support structure and a viewing space to permit imaging of a sample on the flow cell.

3. The system of claim 1, further comprising the fluidic device, the fluidic device further comprising a housing, wherein the flow cell includes an inlet port and an outlet port and a flow channel extending therebetween, the flow cell to hold a sample; and

wherein the housing includes a reception space to receive the flow cell, the reception space being sized and shaped to permit the flow cell to move in at least one direction relative to the housing.

4. The system of claim 3, wherein the fluidic device further comprises a gasket coupled to the housing, the gasket having inlet and outlet passages and comprising a compressible material, the gasket being positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively.

5. The system of claim 3, further comprising an identification transmitter coupled to the housing.

6. The system of claim 5, wherein the identification transmitter comprises an RFID tag.

7. The system of claim 1, wherein the support structure comprises a reference surface and wherein the device holder further comprises an alignment assembly to press the fluidic device against the reference surface in a direction in a XY plane such that the fluidic device is held against the reference surface in a fixed position with respect to the support structure.

8. The system of claim 7, wherein the reference surface is a first reference surface, the support structure further comprises a second reference surface, the first reference surface stopping movement of the fluidic device in a direction along an X axis when the cover is in a closed position and the second reference surface stopping movement of the fluidic device in a direction along a Y axis when the cover is in the closed position.

9. The system of claim 8, wherein the support structure comprises a thermal module to transfer thermal energy through the base surface to control a temperature of the flow cell.

10. The system of claim 1, further comprising a multi-port valve to selectively flow different fluids to the fluidic device, wherein each sipper tube of the array of sipper tubes is in fluid communication with the multi-port valve to selectively flow a corresponding fluid stored in a corresponding component well of the reaction component tray to the fluidic device.

11. The system of claim 1, further comprising the reaction component tray comprising a plurality of component wells storing a plurality of fluids, wherein the plurality of fluids comprises a polymerase, modified nucleotides, or a cleavage mix, wherein the drive motor is to move the array of sipper tubes between a withdrawn level and a deposited level, the distal portions of the sipper tubes of the array of sipper tubes inserted into the component wells to remove fluid therefrom when at the deposited level, and the distal portions of the sipper tubes of the array of sipper tubes completely removed from the reaction component tray at the withdrawn level.



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12. The system of claim 1, wherein the fluid storage system comprises a guide plate, wherein the distal portions of the array of sipper tubes extend through corresponding openings of the guide plate.

13. The system of claim 1, wherein the fluid storage system comprises a sensor to determine a level of the transport platform and wherein when the sensor identifies the level of the transport platform not reaching a threshold level, the system generates an alert indicative that the reaction component tray is not ready for removal.

14. A system comprising:

an optical system comprising an excitation light source, an imaging detector, and an optical train;

a device holder to orient a fluidic device for imaging by the optical system, the fluidic device comprising a flow cell, the device holder comprising:

a support structure including a loading region to receive the fluidic device, the loading region including a base surface to have the fluidic device positioned thereon,

a rotatable cover that is coupled to the support structure and moveable about an axis between an open position and a closed position, the cover movable to the open position to permit the fluidic device to be inserted into and removed from the loading region and movable to the closed position to secure the fluidic device within the loading region for imaging, and

a plurality of alignment members positioned on the support structure to facilitate positioning of the fluidic device within the loading region, wherein each alignment member comprises a reference surface to limit movement of the fluidic device in a direction in an XY-plane;

a fluid storage system comprising:

an enclosure having a cavity, a door openable to provide access to the cavity, a transport platform holding an array of sipper tubes, each sipper tube of the array of sipper tubes including a distal portion positioned to be inserted into a component well of a reagent tray received within the cavity, and

a drive motor operatively coupled to the transport platform, the transport platform moveable by the drive motor to position the array of sipper tubes at least partially within the cavity; and

a casing enclosing the optical system, the device holder, and the fluid storage system therein.

15. The system of claim 14, wherein the fluidic device is pressed against at least one of the plurality of alignment members when the cover is in the closed position such that the fluidic device is held against the at least one of the plurality of alignment members in a fixed position with respect to the support structure.

16. The system of claim 14, wherein the device holder further comprises an alignment assembly comprising a moveable locator arm, the moveable locator arm presses the fluidic device against at least one of the plurality of alignment members such that the fluidic device is held against the at least one of the plurality of alignment members in a fixed position with respect to the support structure.

17. A system comprising:

an optical system comprising an excitation light source, an imaging detector, and an optical train;

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a device holder to orient a fluidic device for imaging by the optical system, the fluidic device comprising a flow cell, the device holder comprising:

a support structure including a loading region to receive the fluidic device, the loading region including a base surface to have the fluidic device positioned thereon,

a rotatable cover that is coupled to the support structure and moveable about an axis between an open position and a closed position, the cover movable to the open position to permit the fluidic device to be inserted into and removed from the loading region and movable to the closed position to secure the fluidic device within the loading region for imaging, the cover comprising a compression arm to press the fluidic device against the support structure and a viewing space to permit imaging of a sample on the flow cell, wherein the cover is biased toward the open position,

a latch to releasably hold the cover in the closed position, and

a plurality of alignment members positioned on the support structure to facilitate positioning of the fluidic device within the loading region, wherein each alignment member comprises a reference surface to limit movement of the fluidic device in a direction in an XY-plane,

a fluid storage system comprising:

an enclosure having a cavity,

a door openable to provide access to the cavity,

a transport platform holding an array of sipper tubes, each sipper tube of the array of sipper tubes including a distal portion oriented for insertion into a component well of a reagent tray received within the cavity, and

a drive motor operatively coupled to the transport platform, the transport platform moveable by the drive motor to position the array of sipper tubes at least partially within the cavity; and

a casing enclosing the optical system, the device holder, and the fluid storage system therein.

18. The system of claim 17, wherein the fluidic device is pressed against at least one of the plurality of alignment members when the cover is in the closed position such that the fluidic device is held against the at least one of the plurality of alignment members in a fixed position with respect to the support structure.

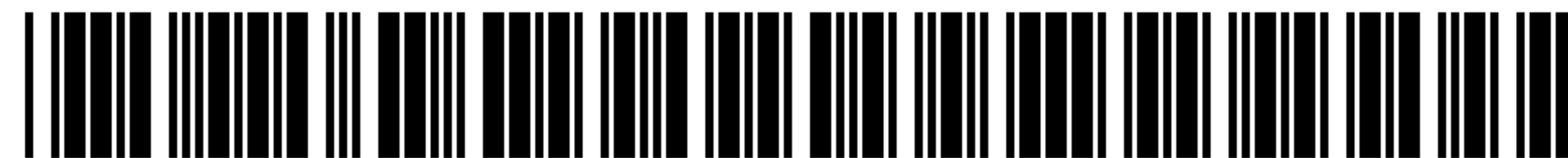
19. The system of claim 17, wherein the device holder further comprises a moveable locator arm, the moveable locator arm pressing the fluidic device against at least one of the plurality of alignment members such that the fluidic device is held against the at least one of the plurality of alignment members in a fixed position with respect to the support structure when the cover is in the closed position.

20. The system of claim 17, further comprising the reagent tray comprising a plurality of component wells storing a plurality of fluids, wherein the plurality of fluids comprises a polymerase, modified nucleotides, or a cleavage mix, wherein the drive motor is to move the array of sipper tubes between a withdrawn level and a deposited level, the distal portions of the sipper tubes of the array of sipper tubes inserted into the component wells to remove fluid therefrom when at the deposited level, and the distal portions of the sipper tubes of the array of sipper tubes completely removed from the reaction component tray at the withdrawn level.



# **EXHIBIT 3**





US008951781B2

(12) **United States Patent**  
**Reed et al.**

(10) **Patent No.:** **US 8,951,781 B2**  
(45) **Date of Patent:** **Feb. 10, 2015**

(54) **SYSTEMS, METHODS, AND APPARATUSES TO IMAGE A SAMPLE FOR BIOLOGICAL OR CHEMICAL ANALYSIS**

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(Continued)

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**C12M 3/00** (2006.01)  
**B01L 9/00** (2006.01)  
**B01L 3/00** (2006.01)

(52) **U.S. Cl.**  
CPC ..... **B01L 9/527** (2013.01); **B01L 3/502715** (2013.01); **B01L 2200/025** (2013.01);

(Continued)

(58) **Field of Classification Search**

CPC ..... B01L 9/527; B01L 3/502715

USPC ..... 435/287.2

See application file for complete search history.

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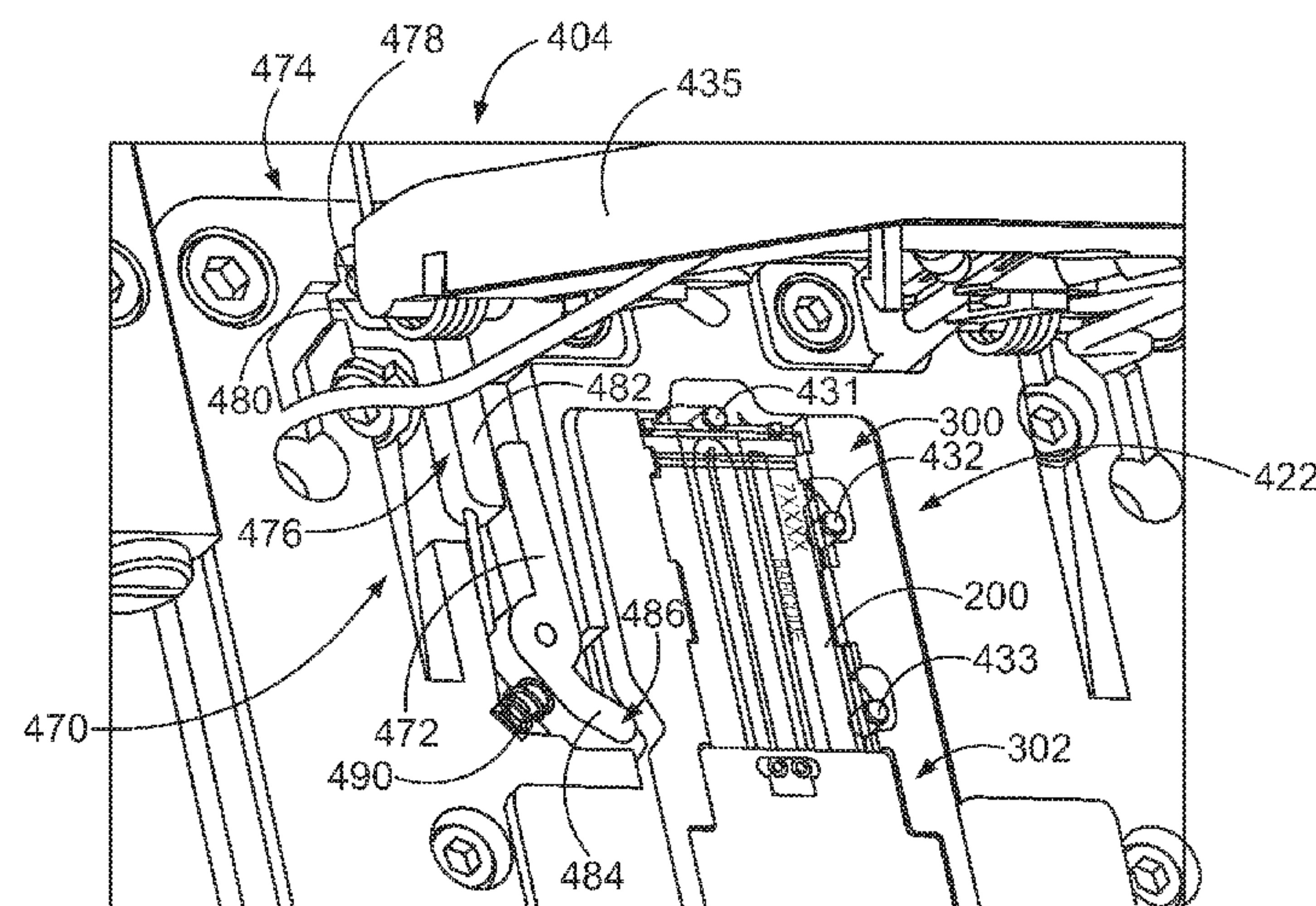
*Primary Examiner* — Jonathan Hurst

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(57) **ABSTRACT**

A fluidic device holder configured to orient a fluidic device. The device holder includes a support structure configured to receive a fluidic device. The support structure includes a base surface that faces in a direction along the Z-axis and is configured to have the fluidic device positioned thereon. The device holder also includes a plurality of reference surfaces facing in respective directions along an XY-plane. The device holder also includes an alignment assembly having an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end away from and toward the reference surfaces. The locator arm is configured to hold the fluidic device against the reference surfaces when the locator arm is in the biased position.

**44 Claims, 39 Drawing Sheets**





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Related U.S. Application Data

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- (52) **U.S. Cl.**  
CPC .... *B01L2200/027* (2013.01); *B01L 2200/0689* (2013.01); *B01L 2300/043* (2013.01); *B01L 2300/0816* (2013.01); *B01L 2300/0877* (2013.01)  
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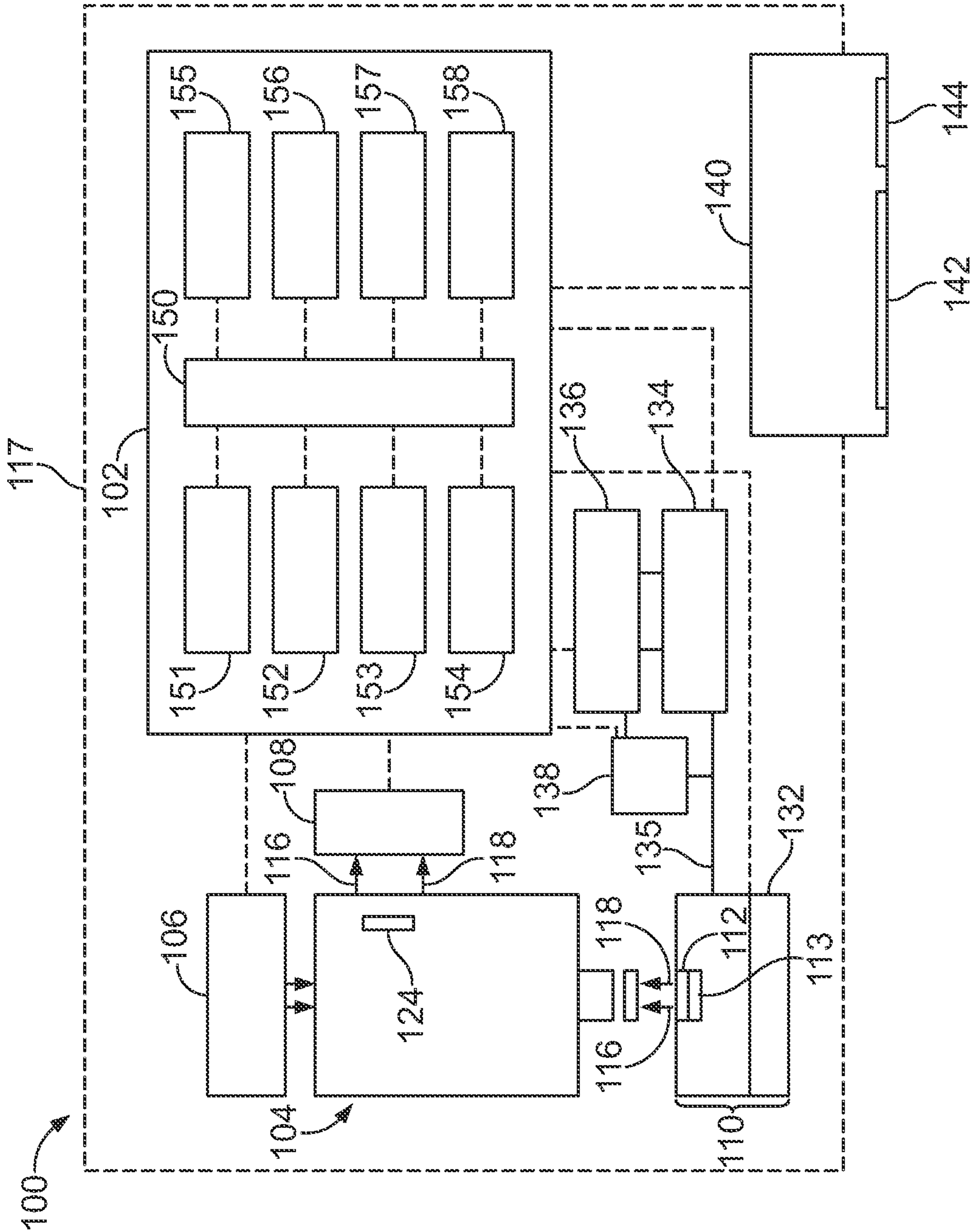


FIG. 1



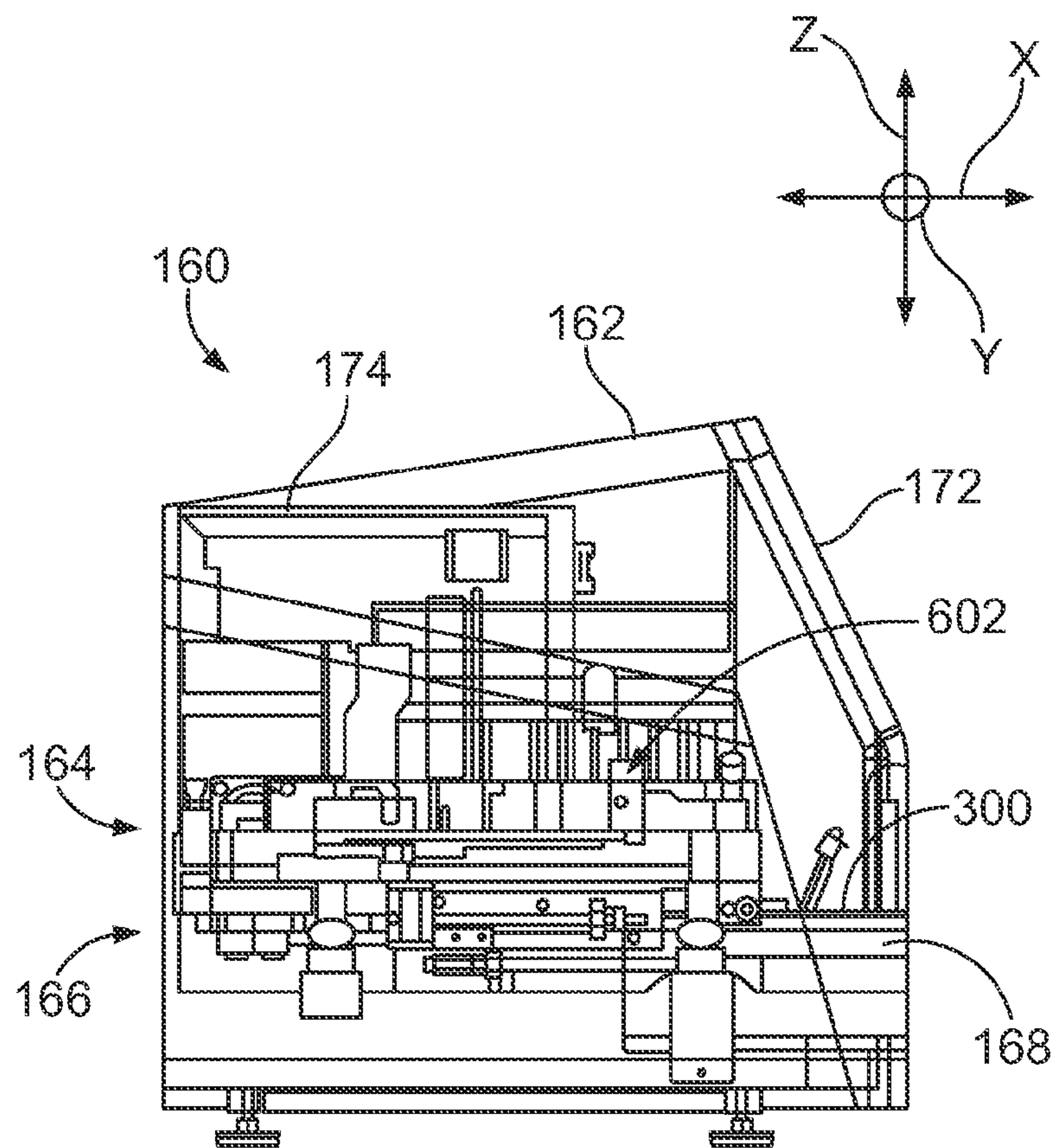


FIG. 2

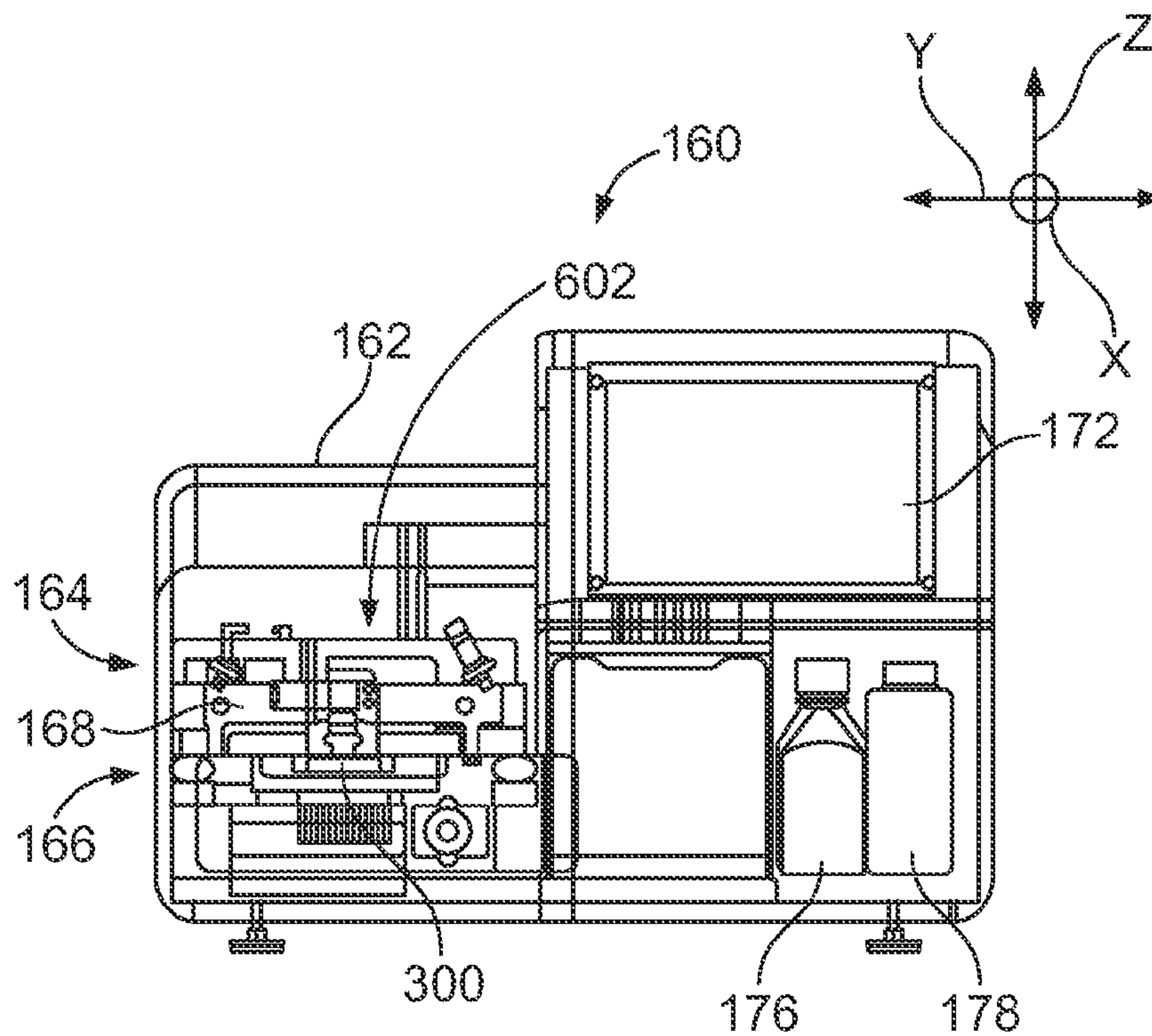


FIG. 3







FIG. 5



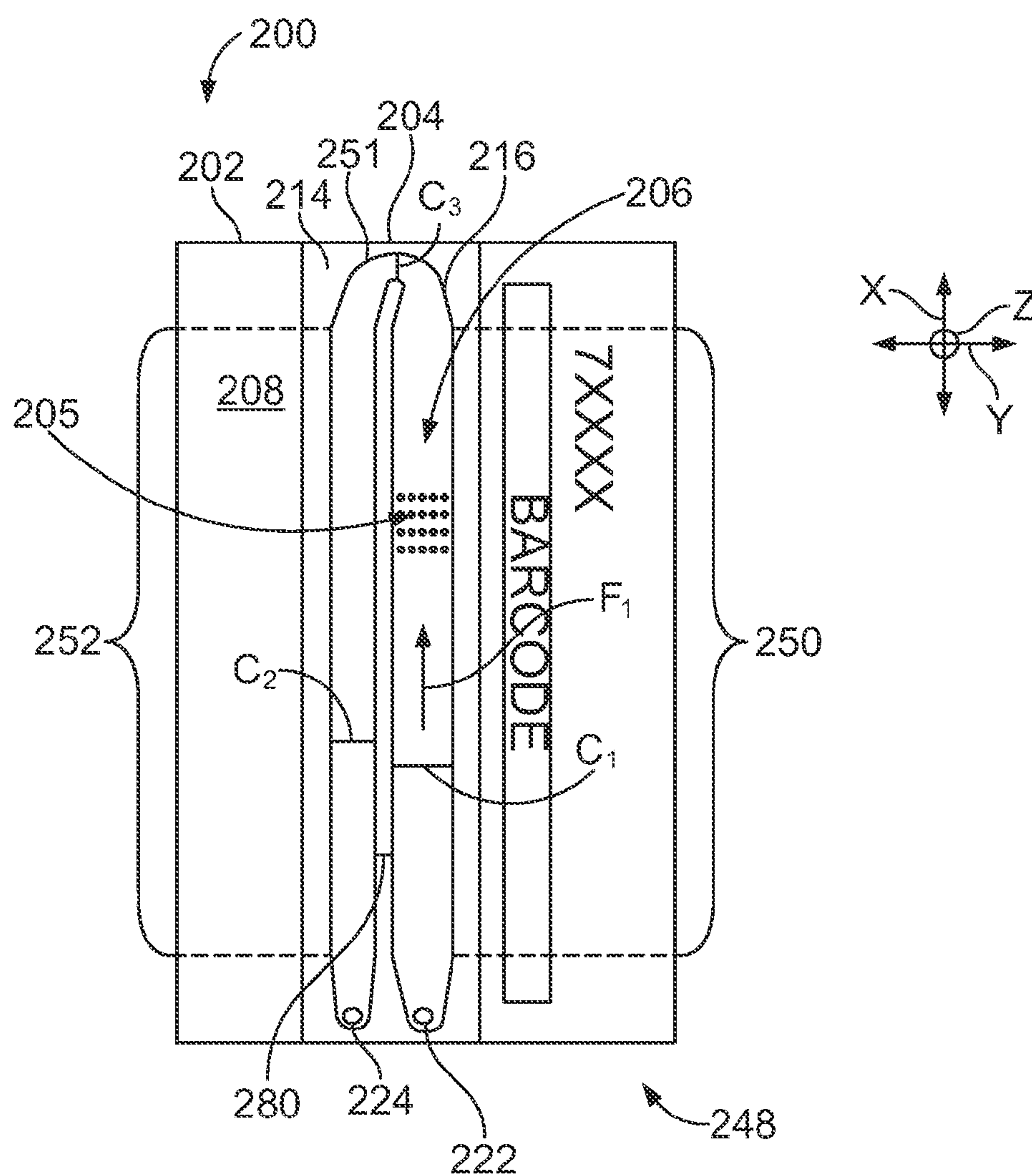
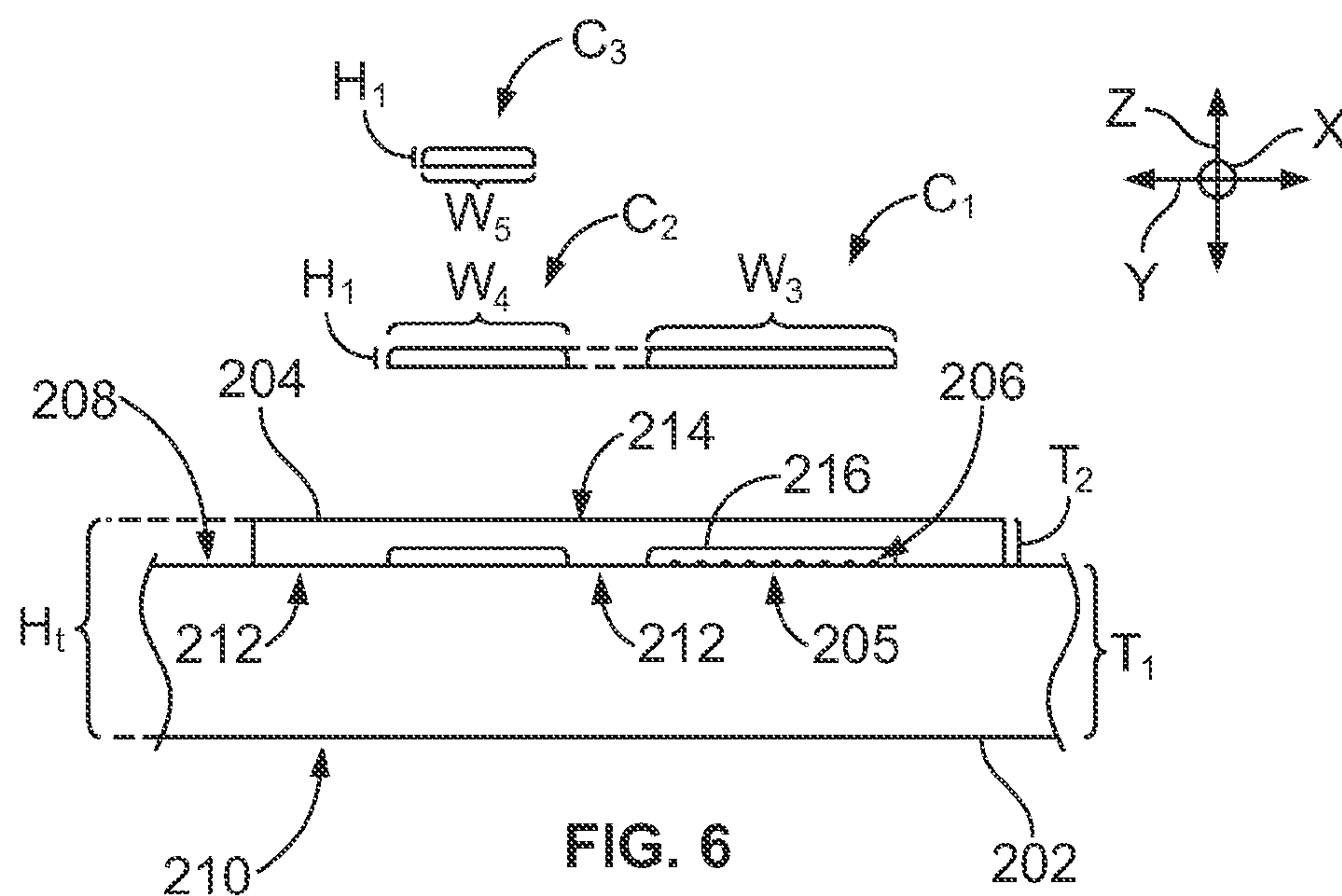


FIG. 7



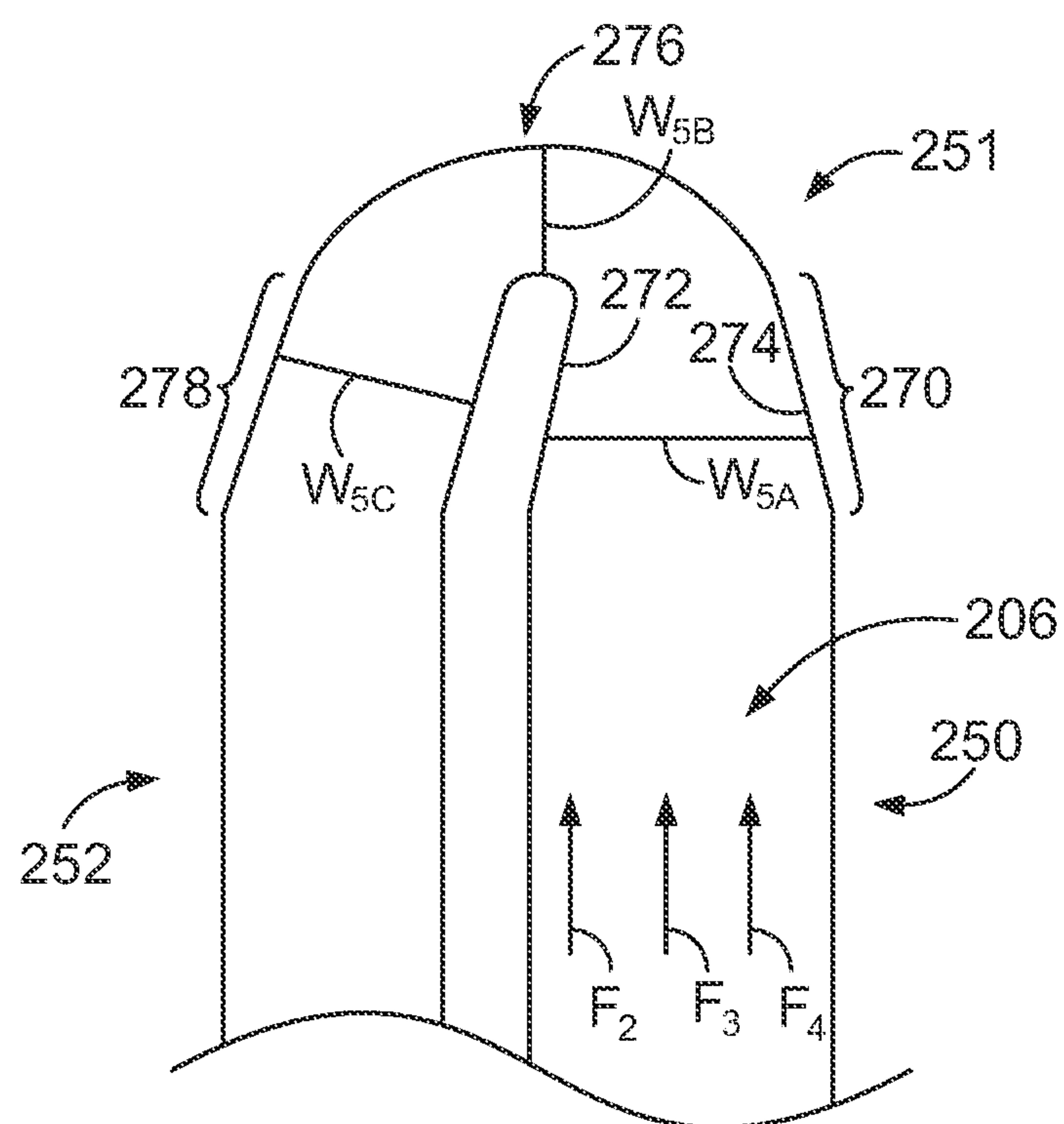


FIG. 8



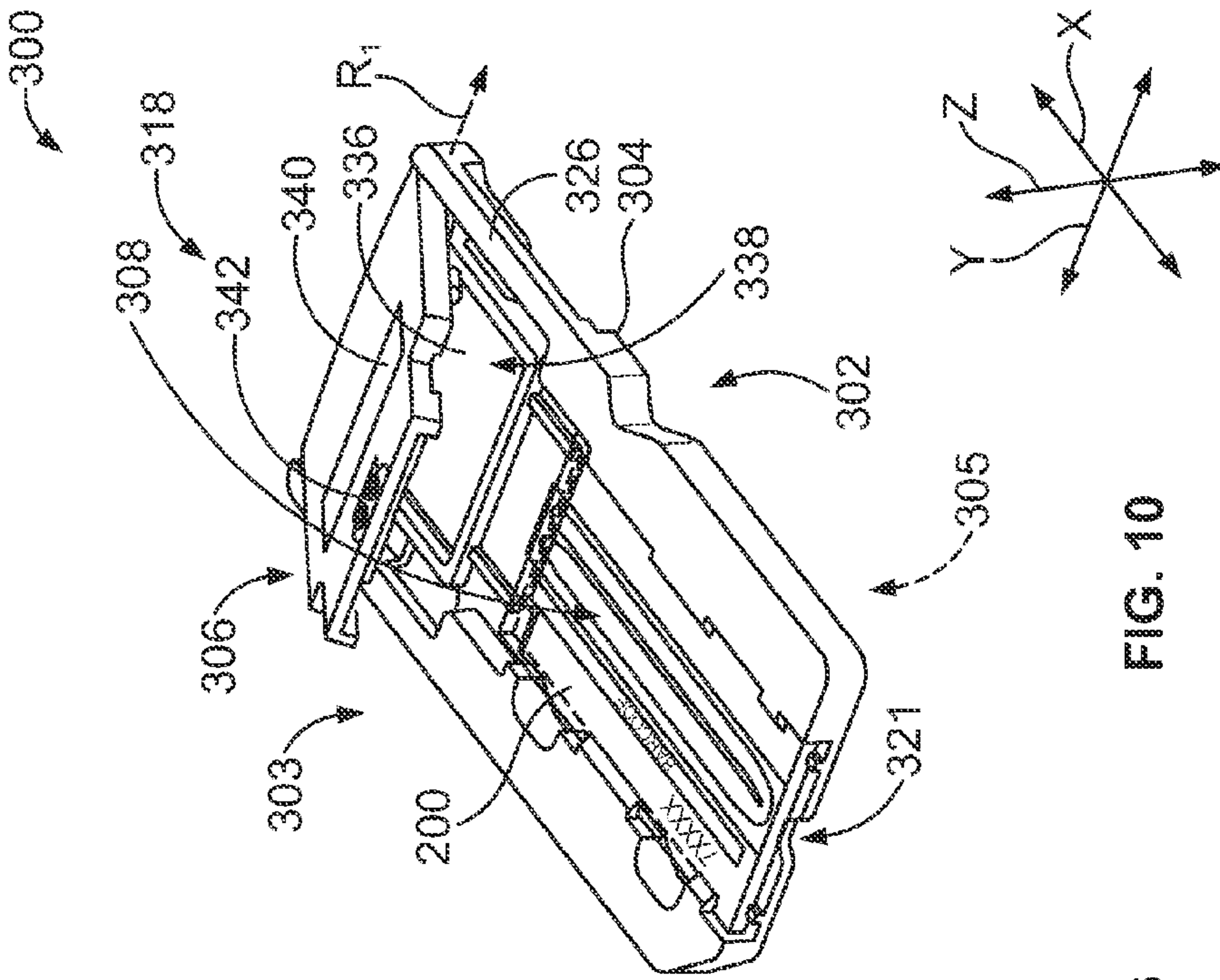


FIG. 10

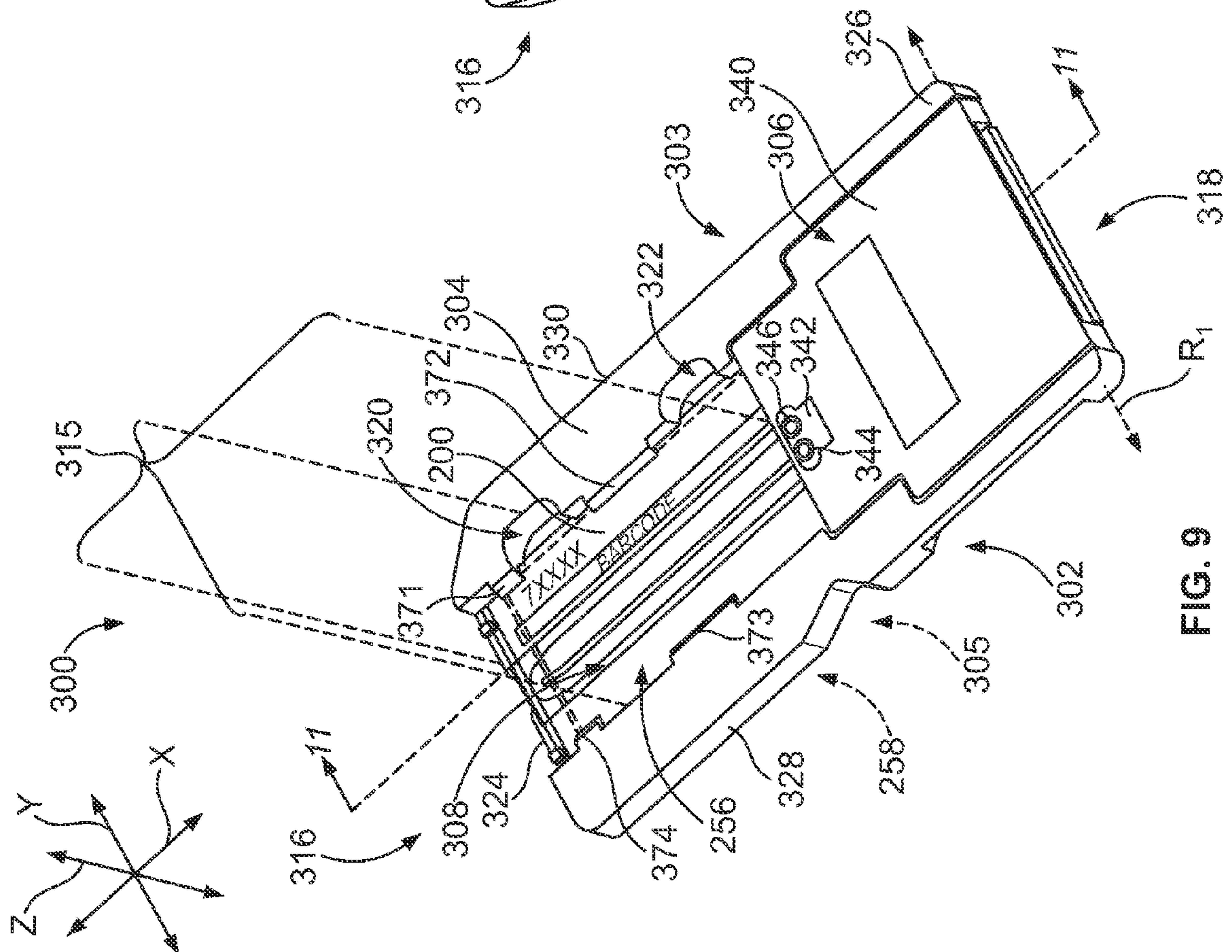
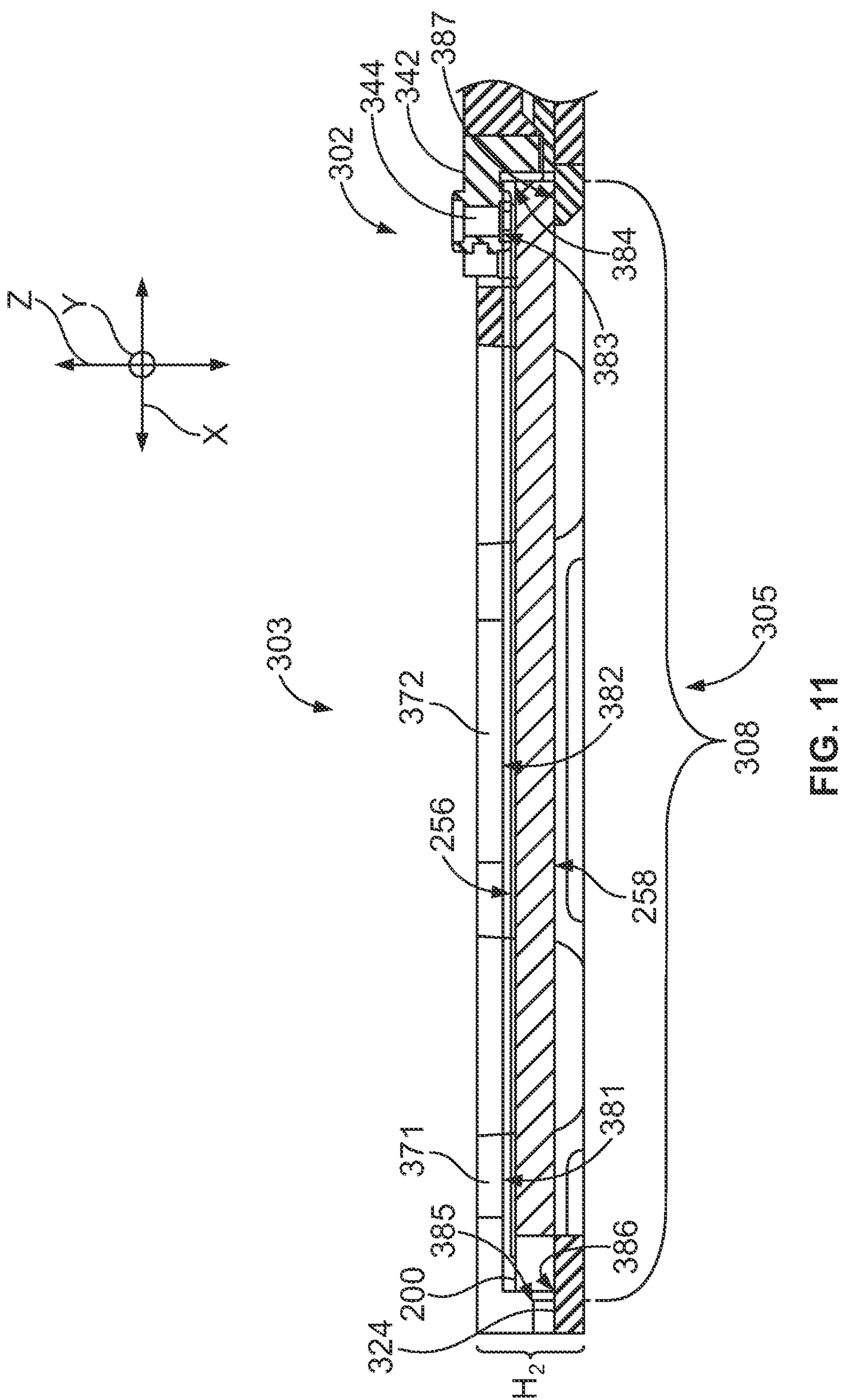


FIG. 9







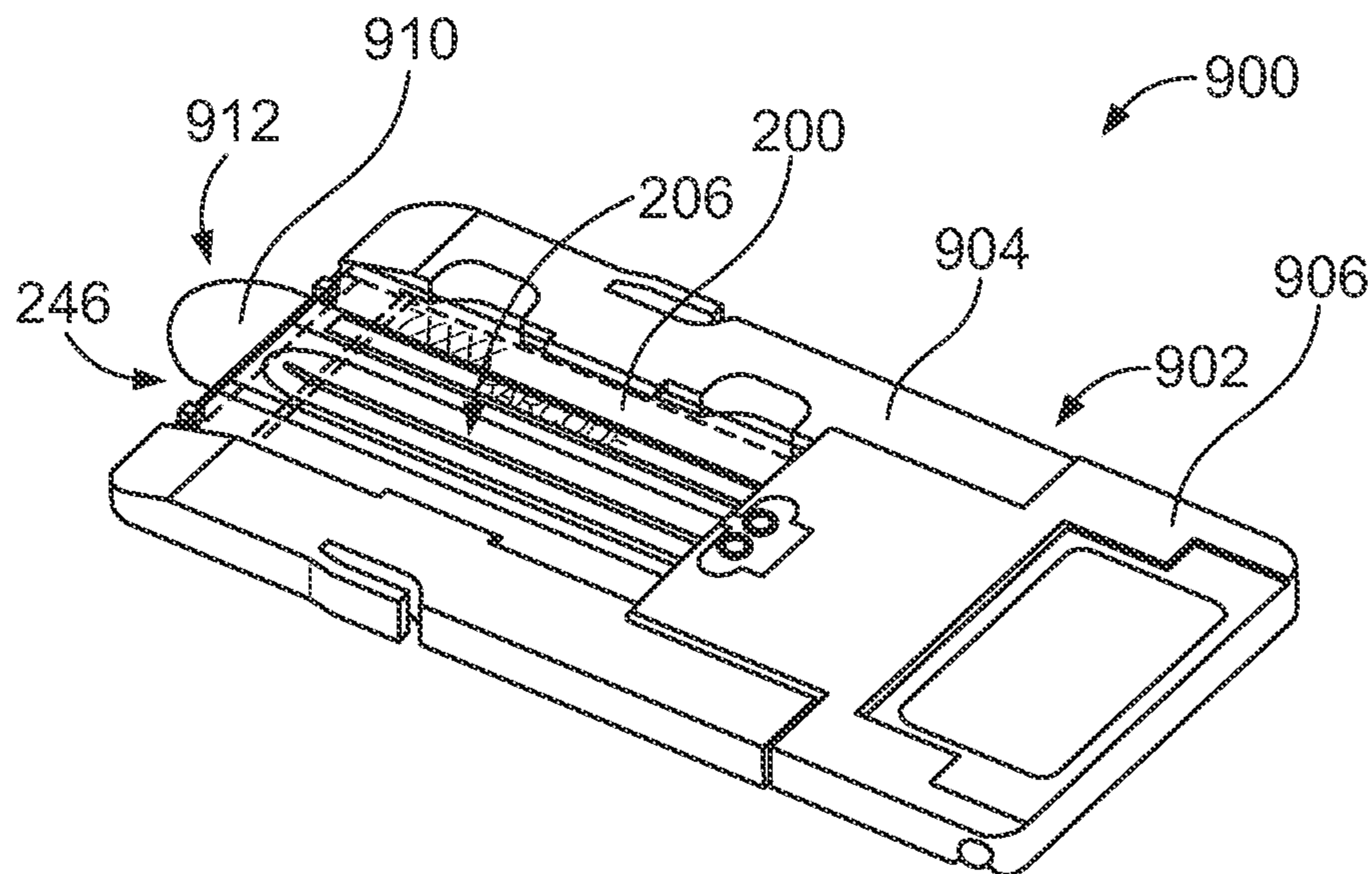


FIG. 12

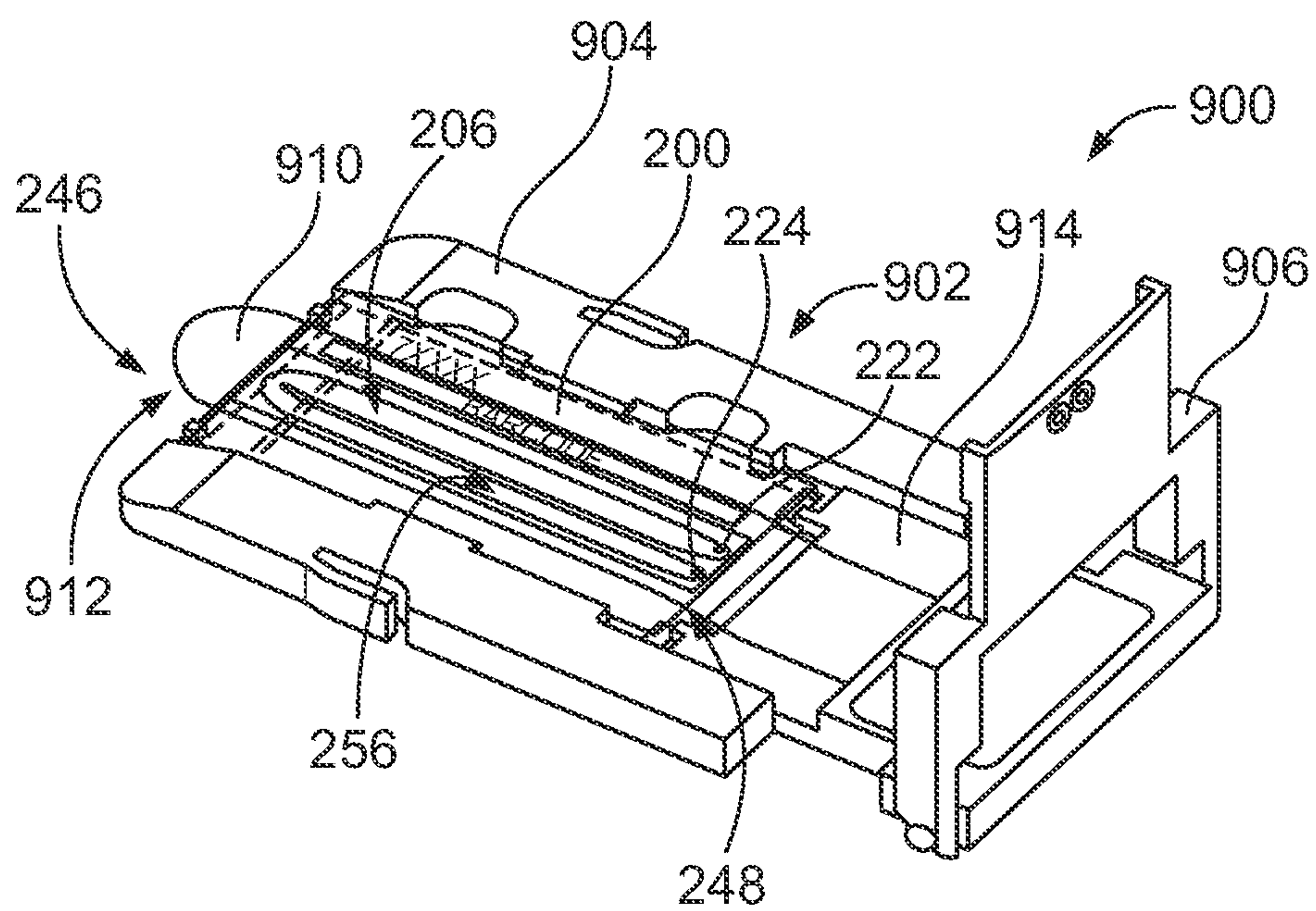
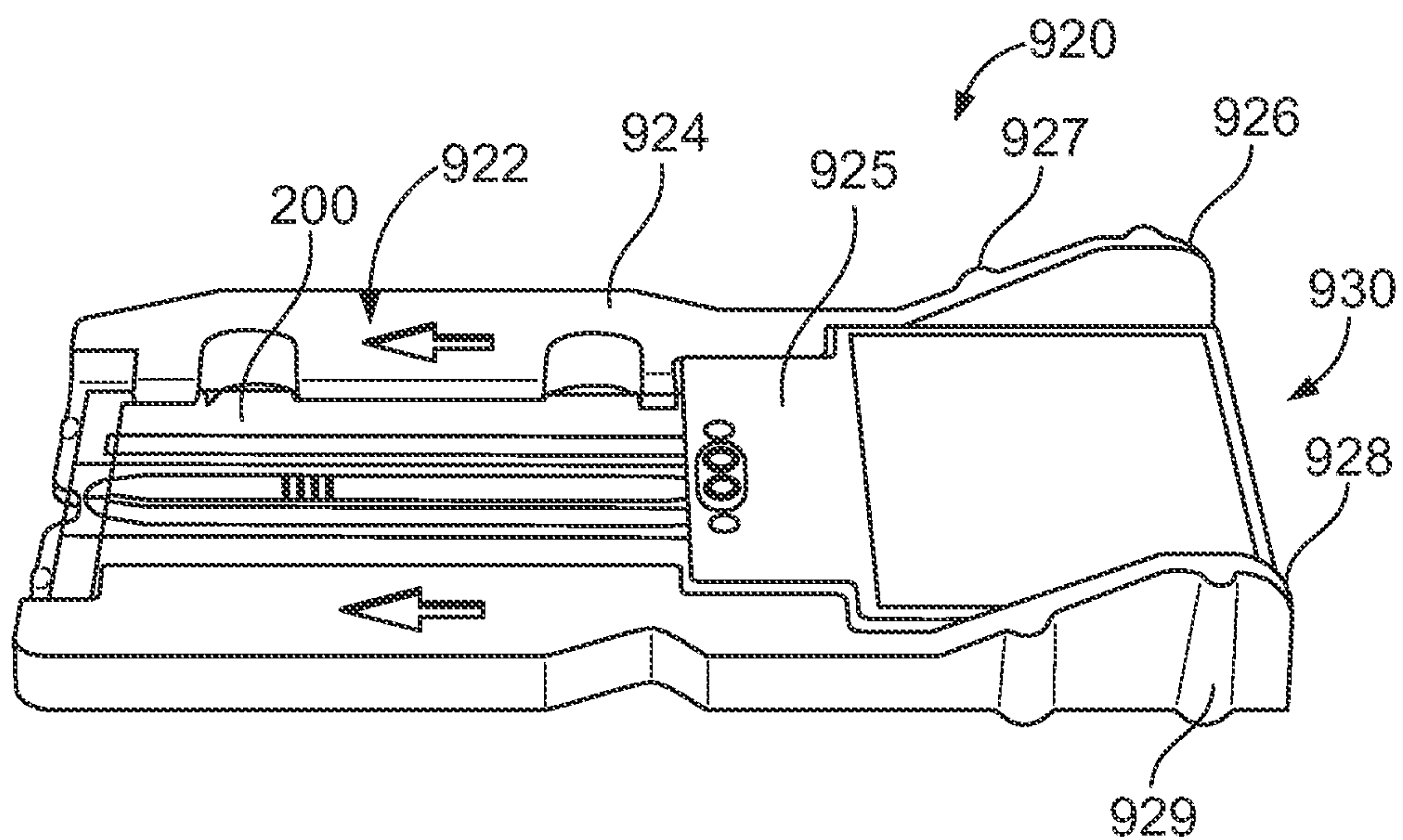
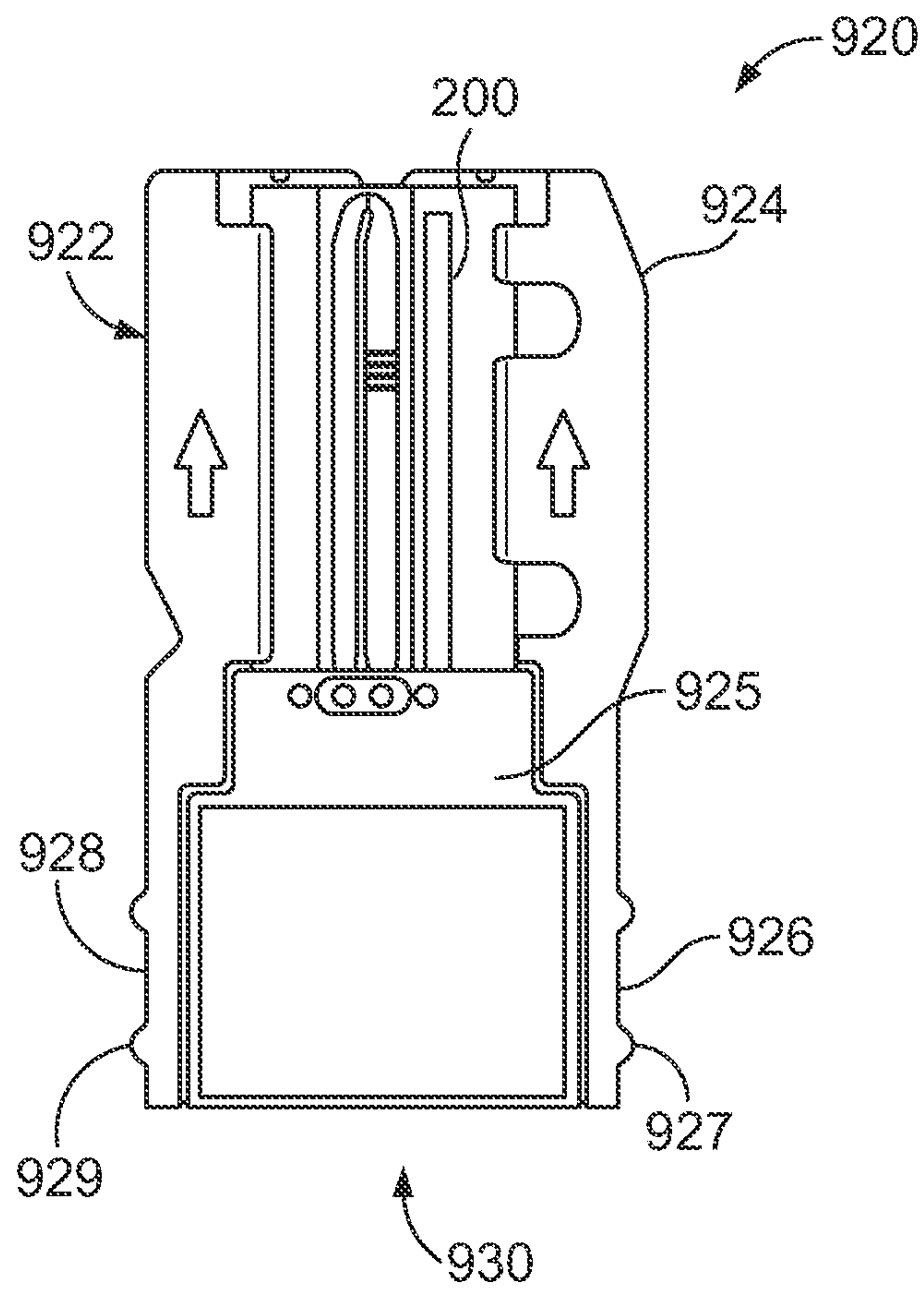


FIG. 13







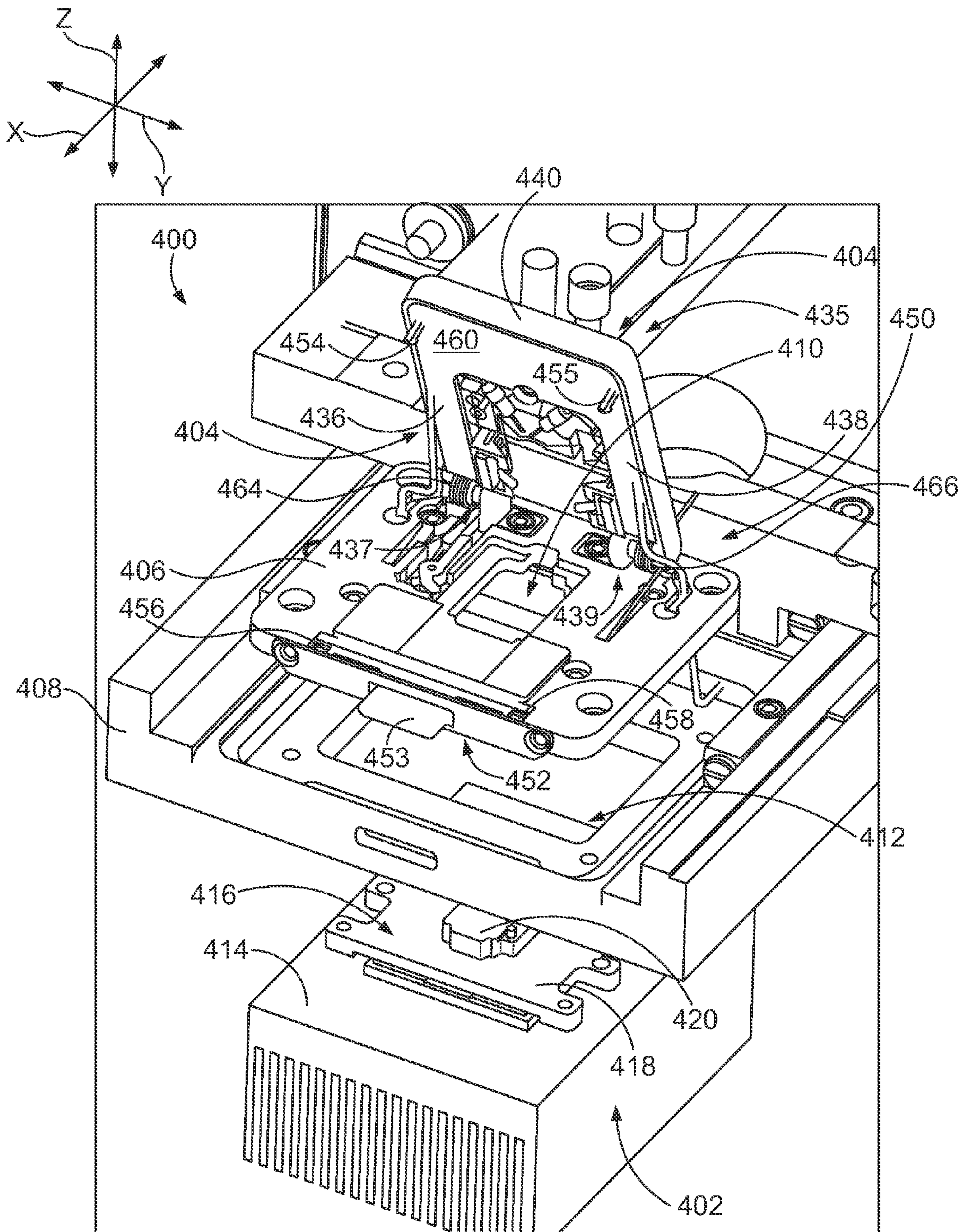
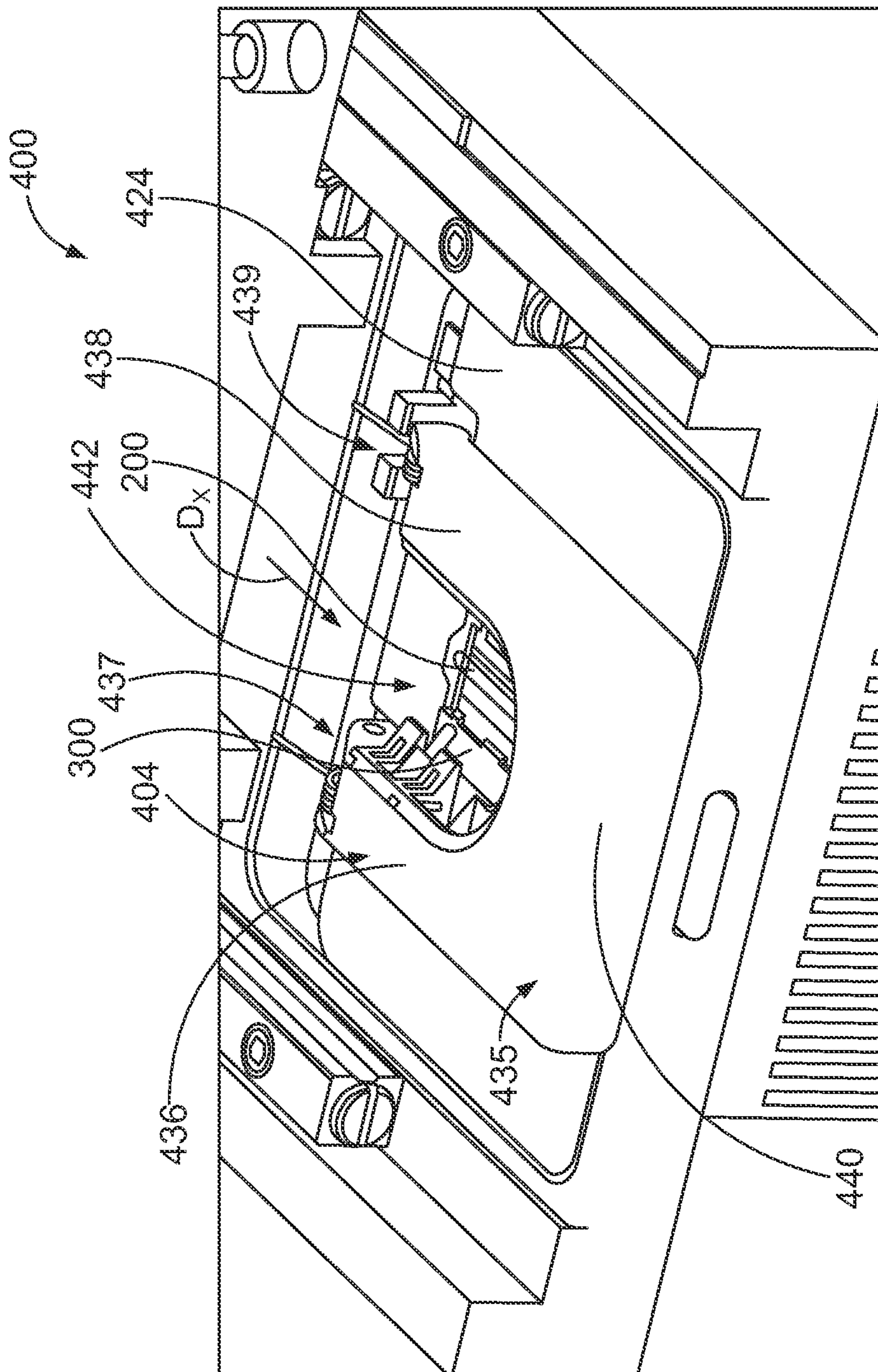


FIG. 16





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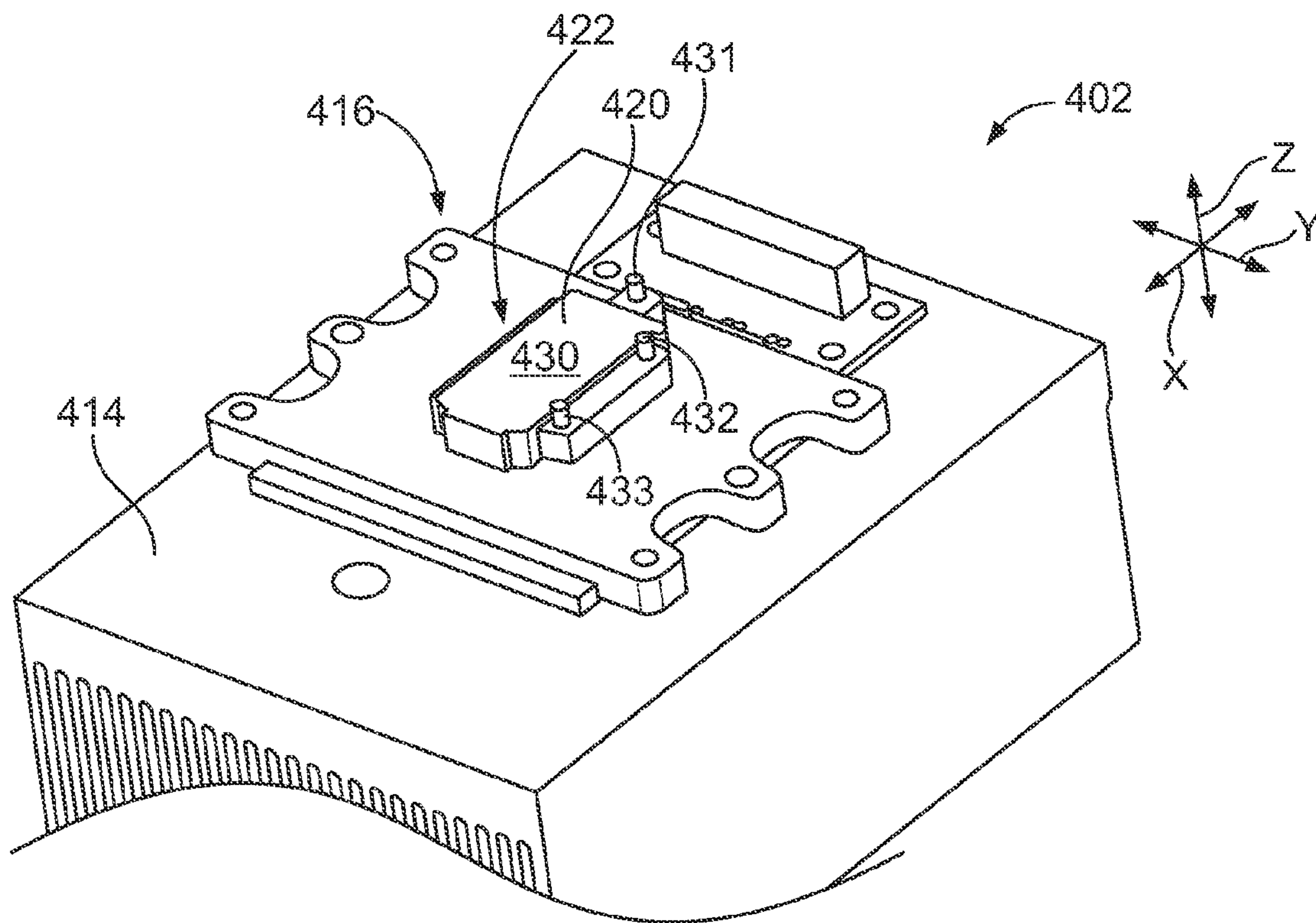


FIG. 18



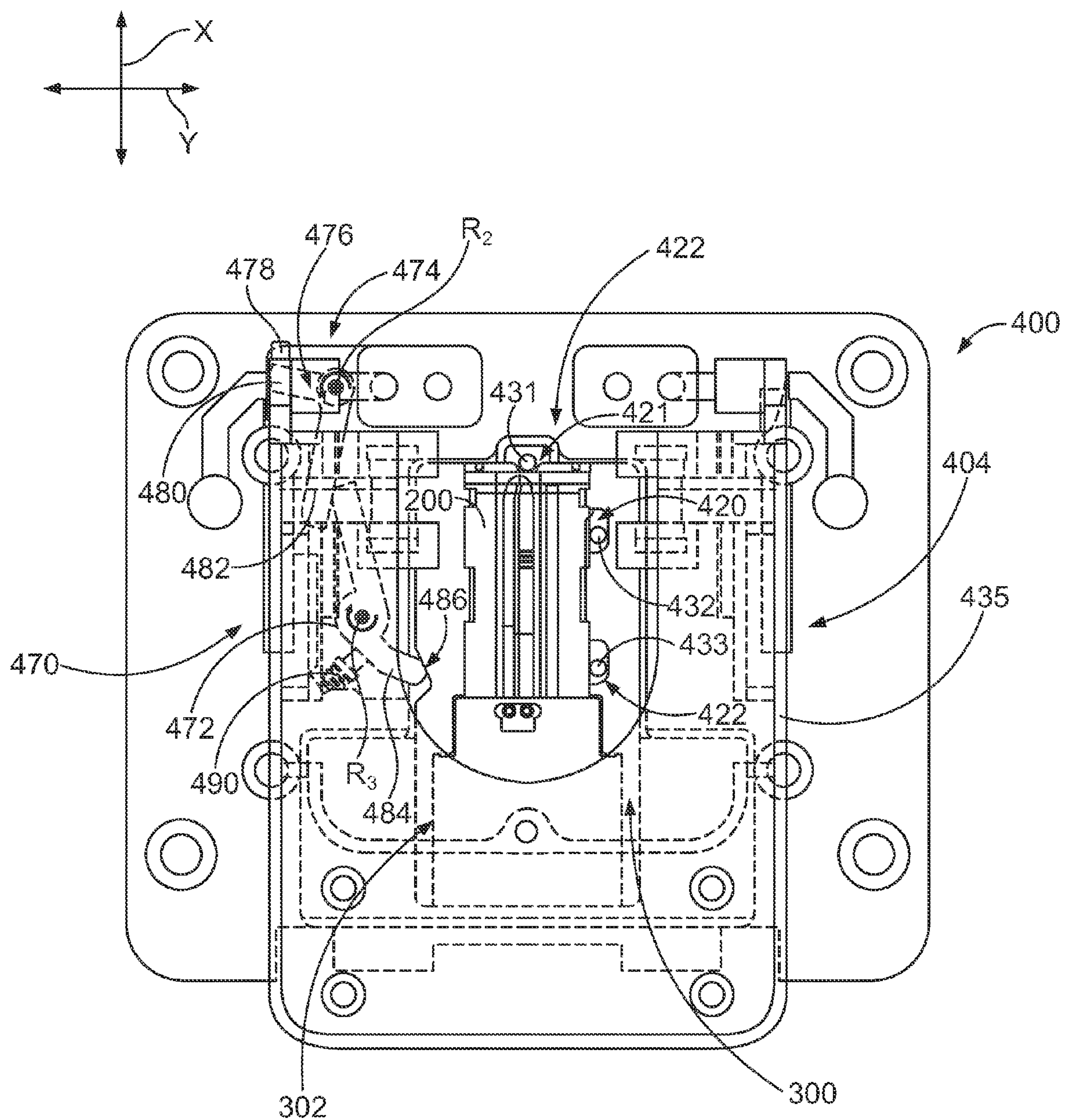


FIG. 19



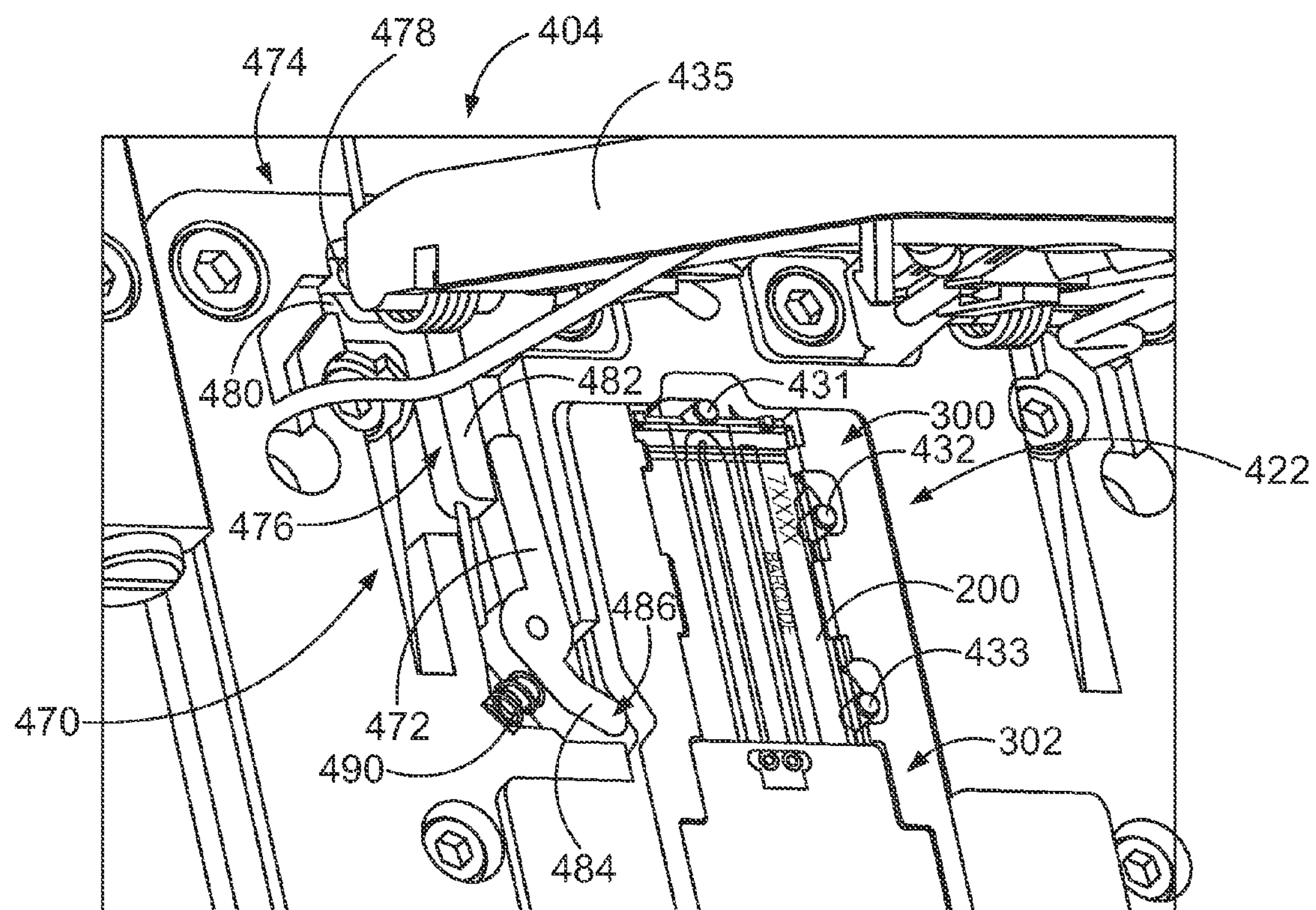


FIG. 20



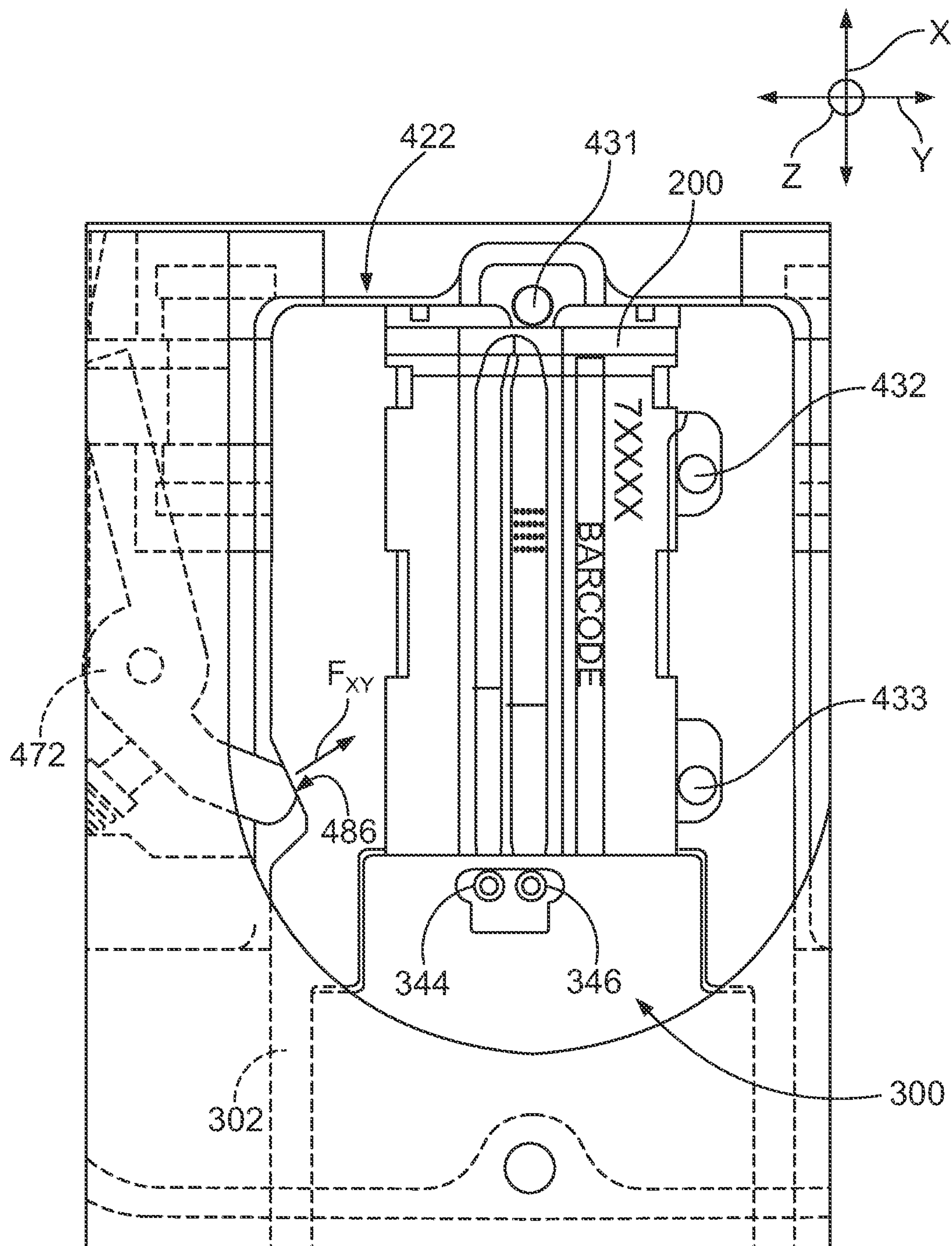


FIG. 21



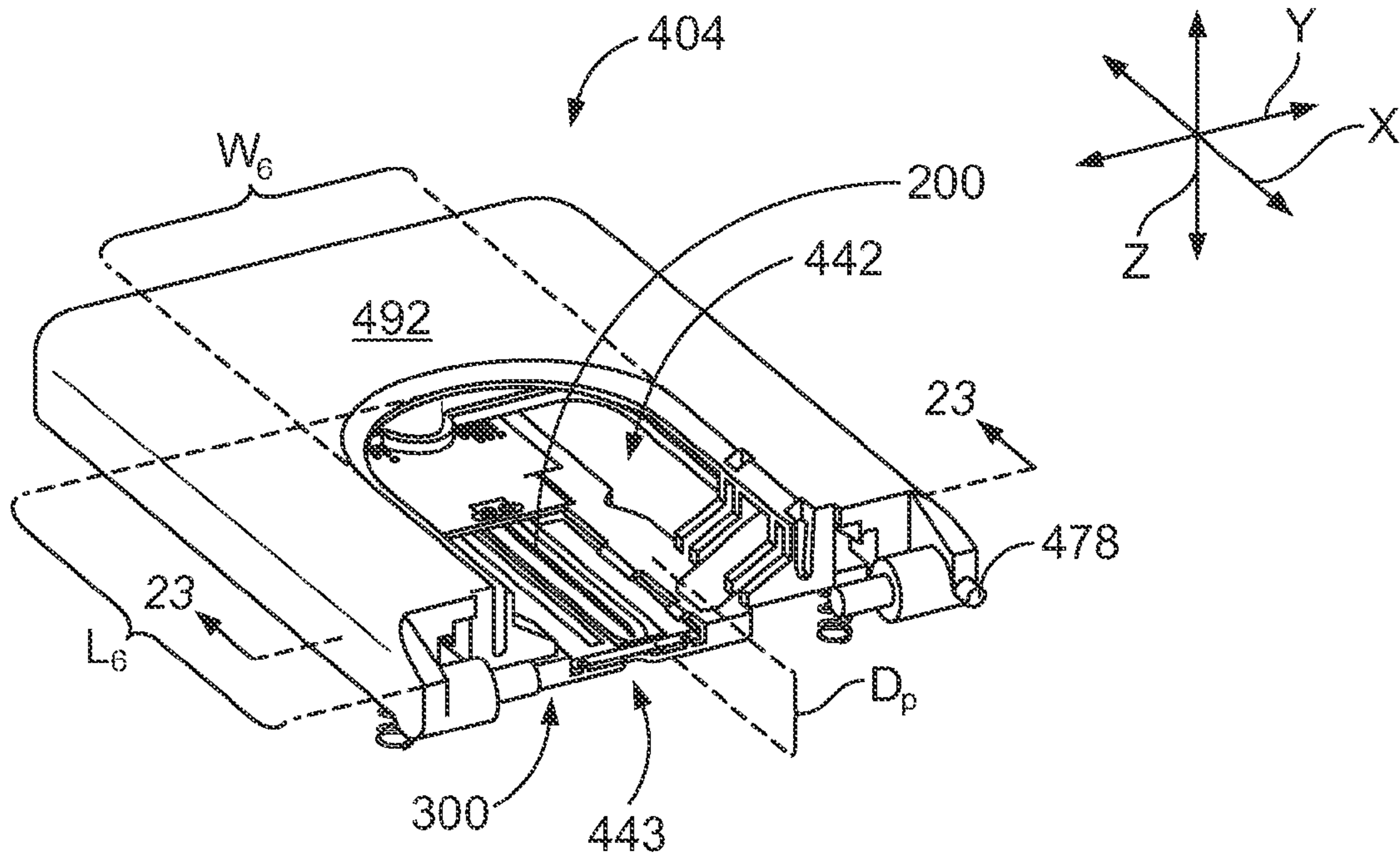


FIG. 22

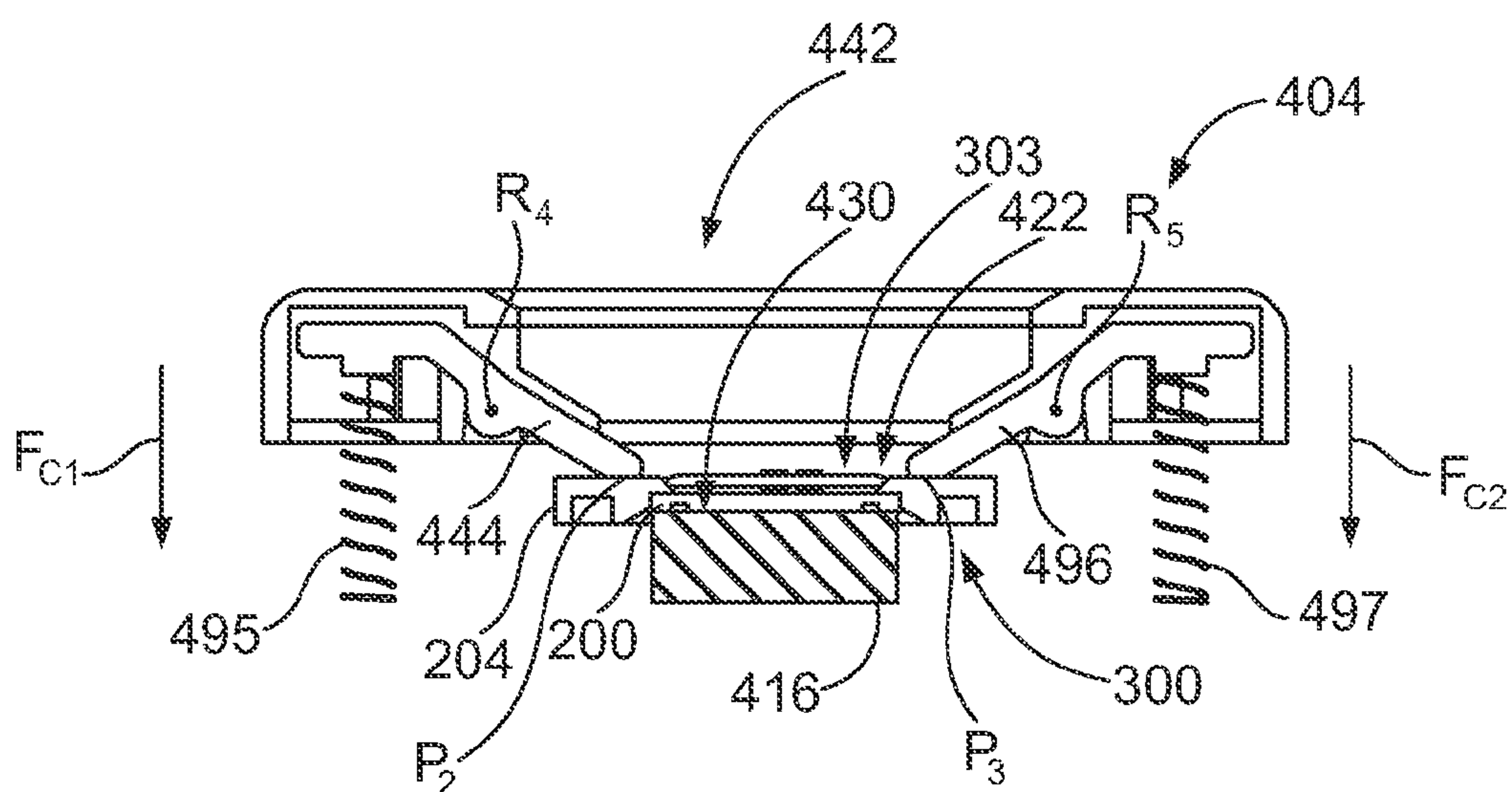


FIG. 23



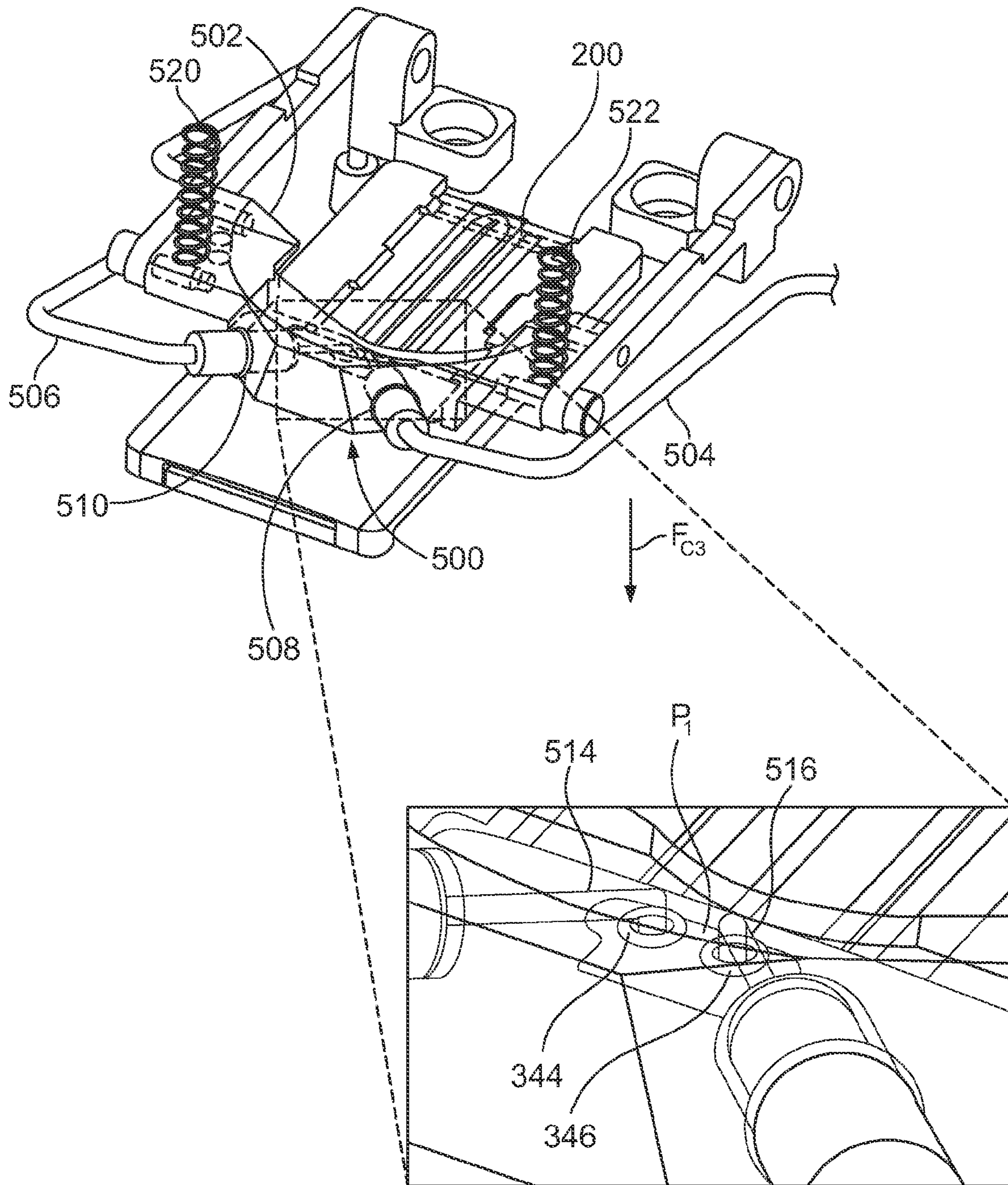


FIG. 24



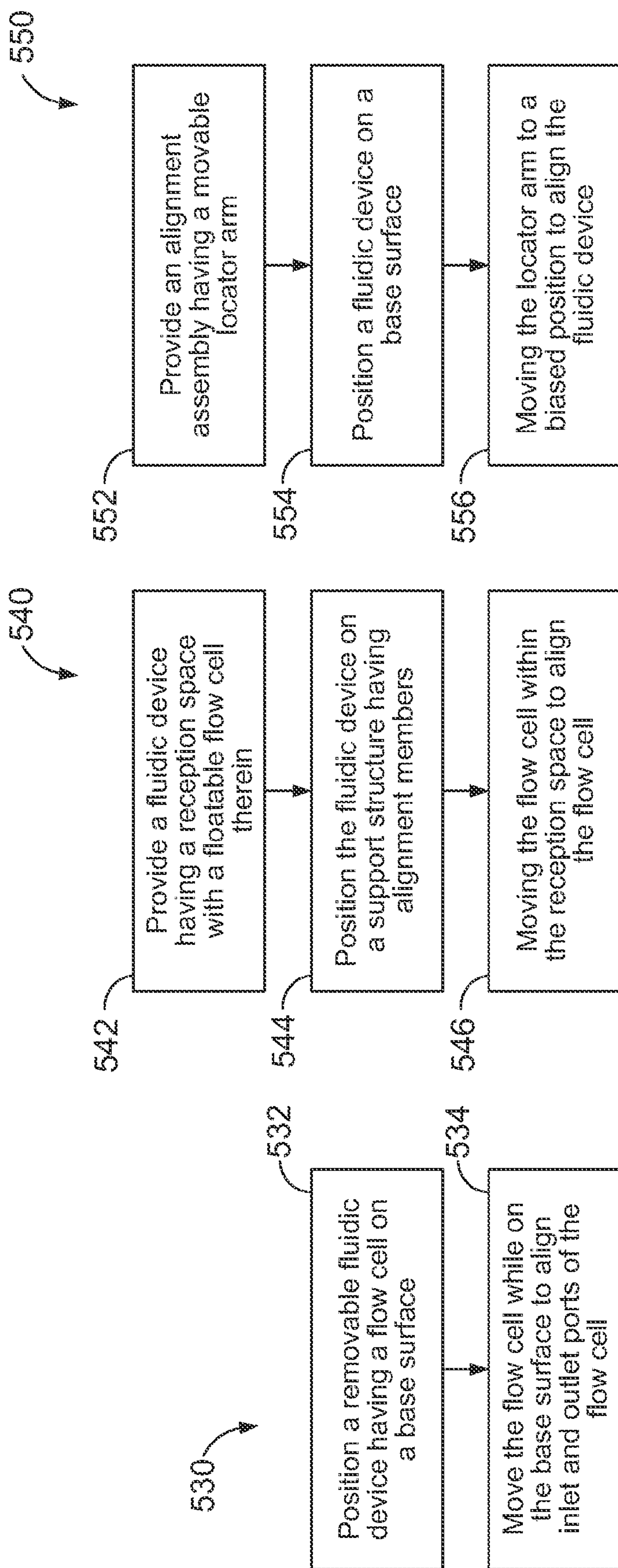


FIG. 25

FIG. 26

FIG. 27



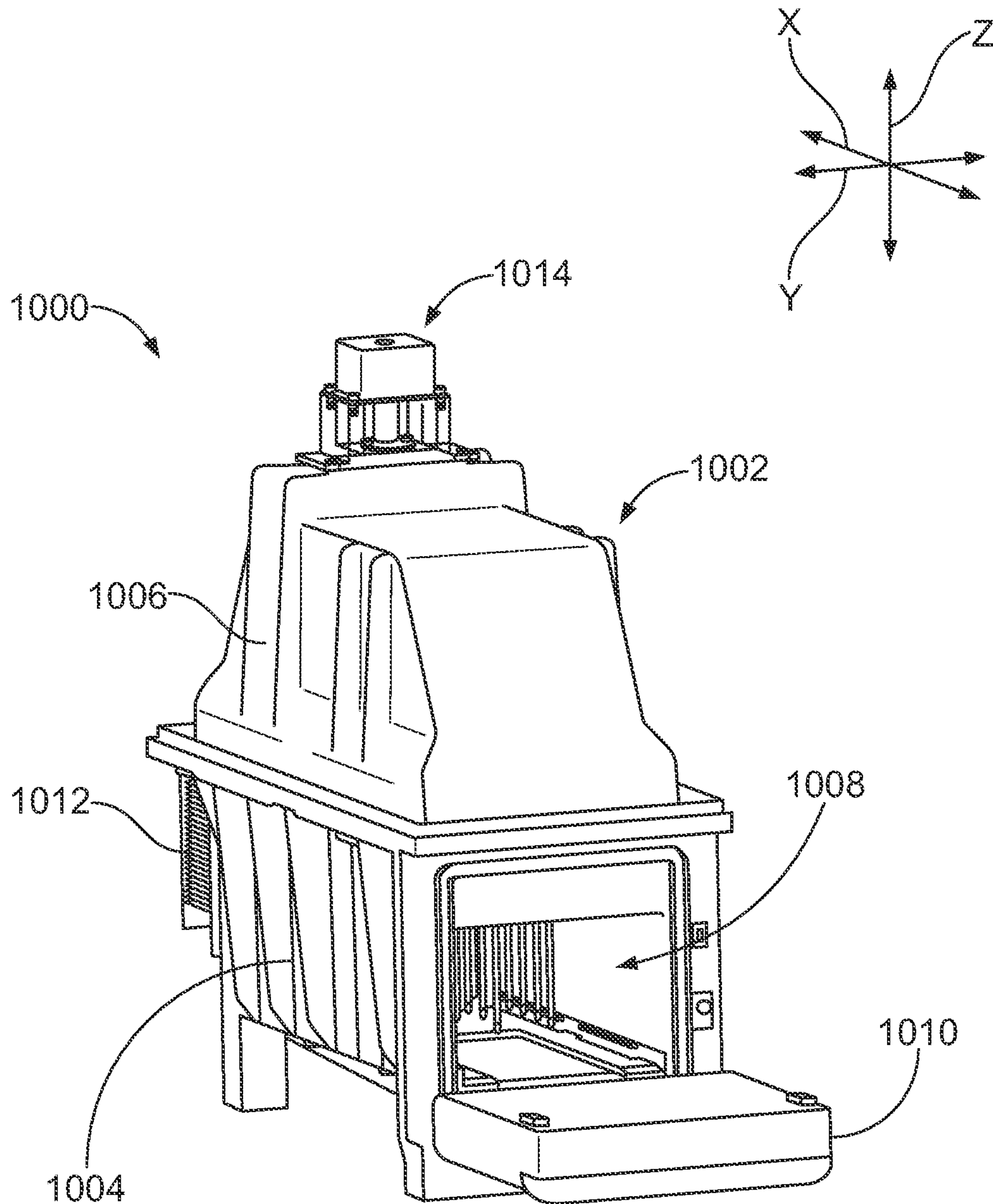


FIG. 28



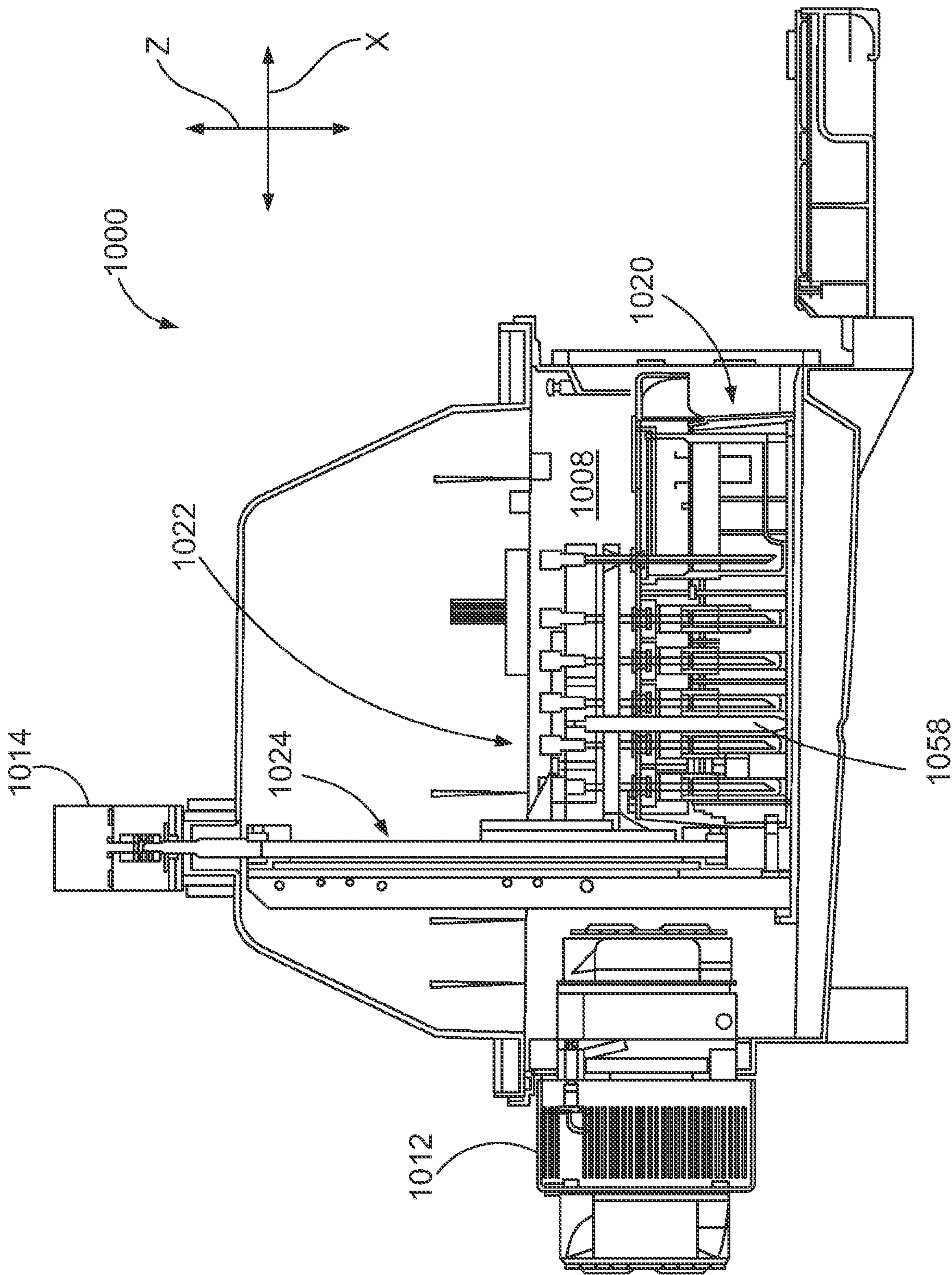


FIG. 29



FIG. 30



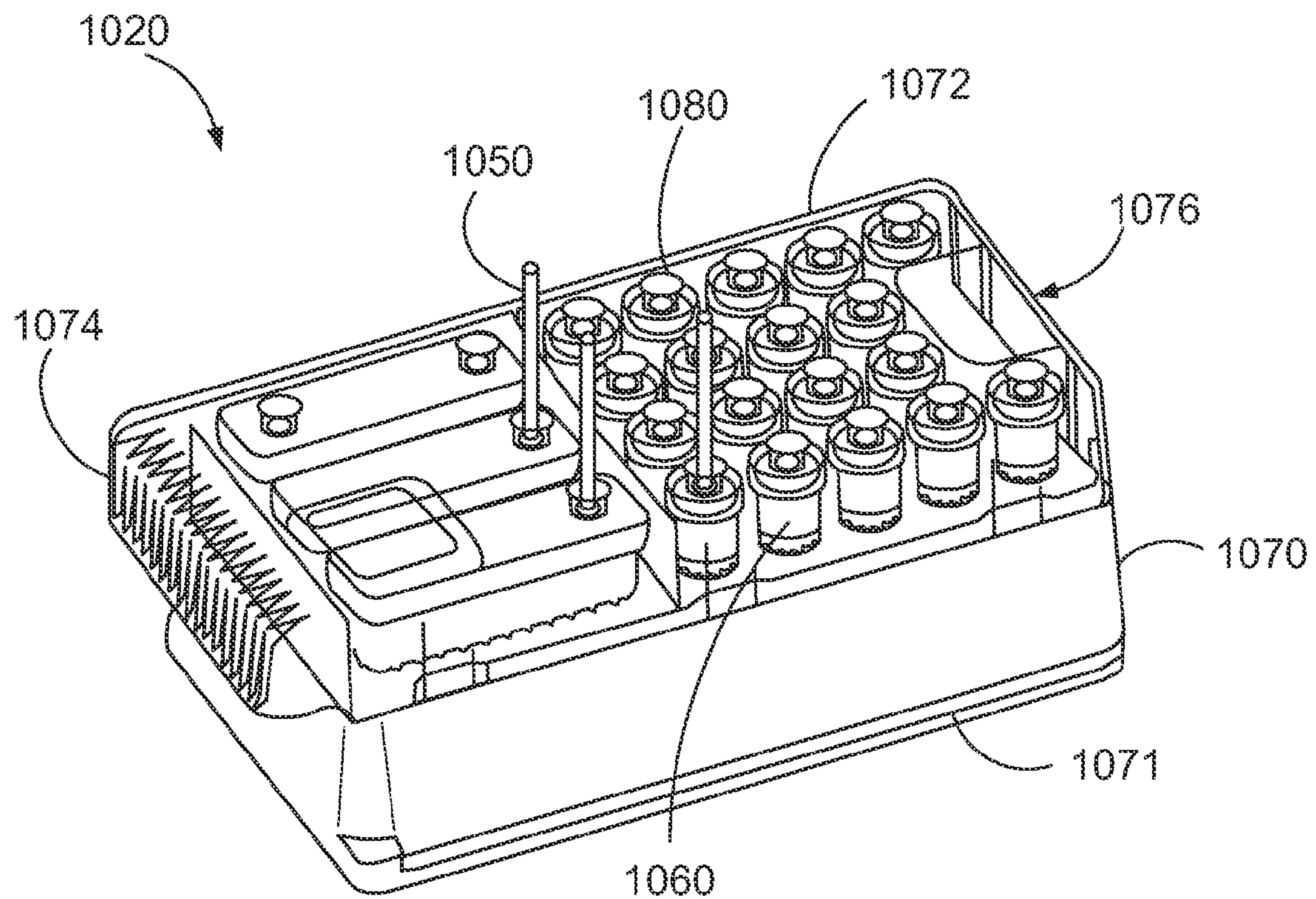


FIG. 31

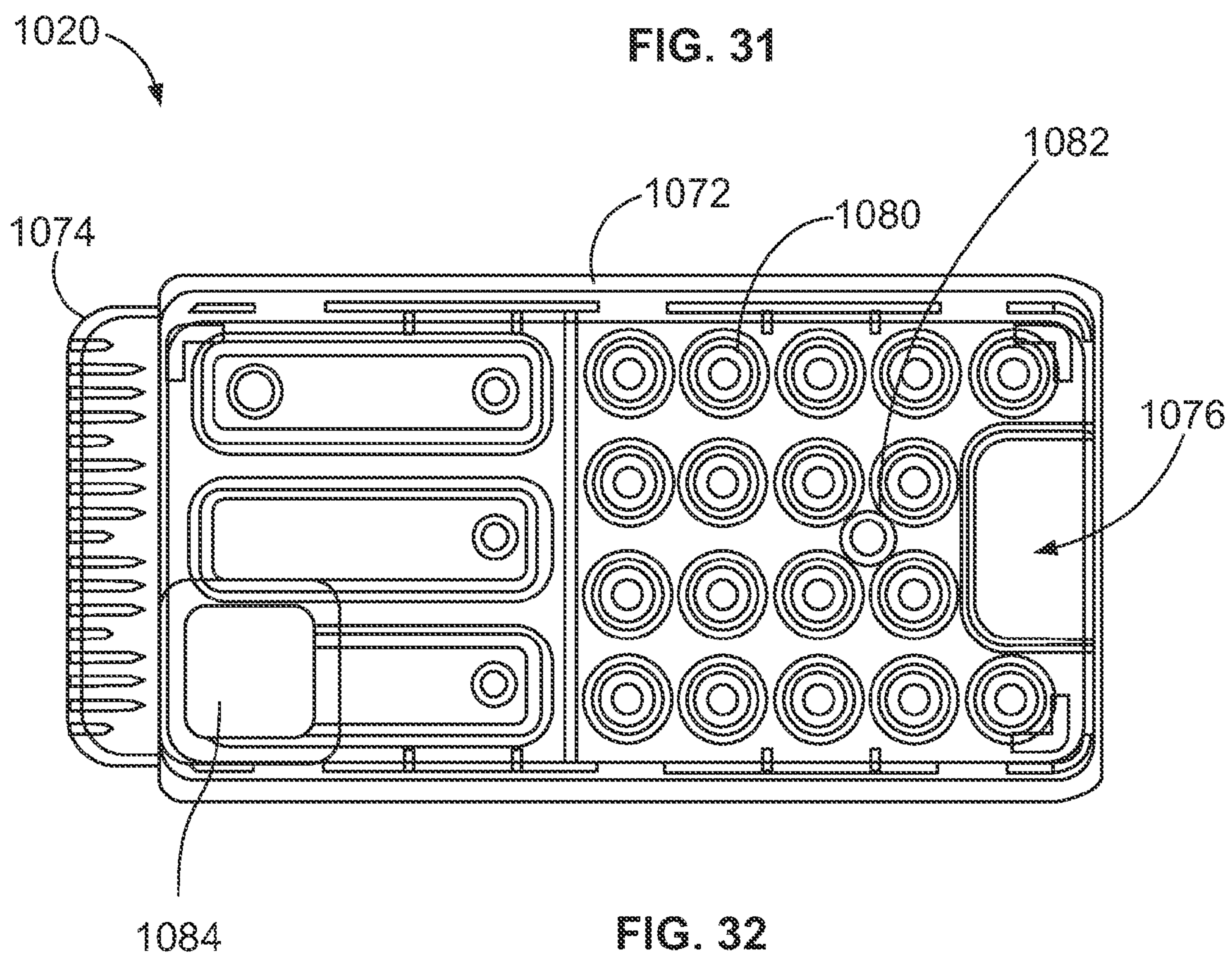


FIG. 32



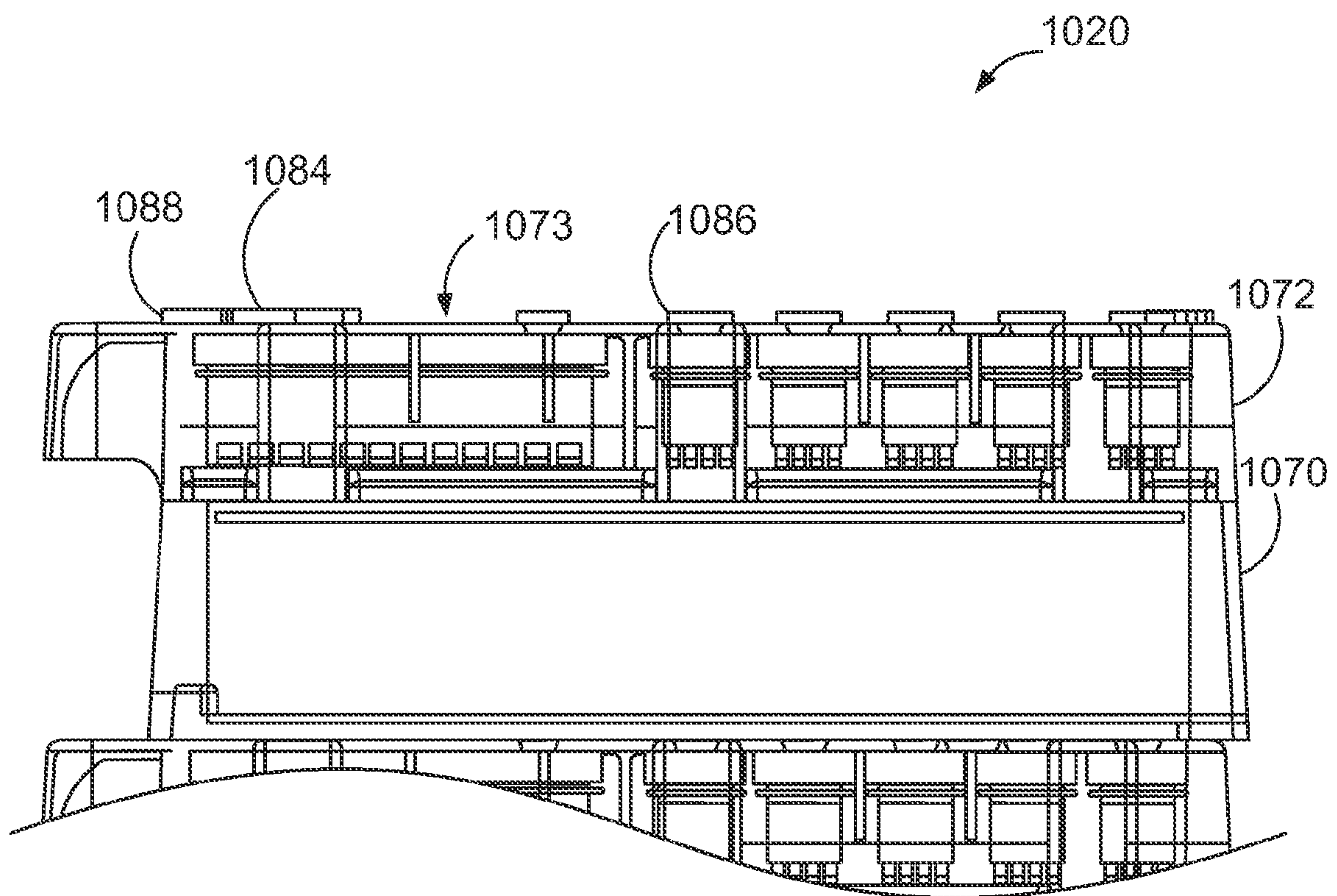


FIG. 33

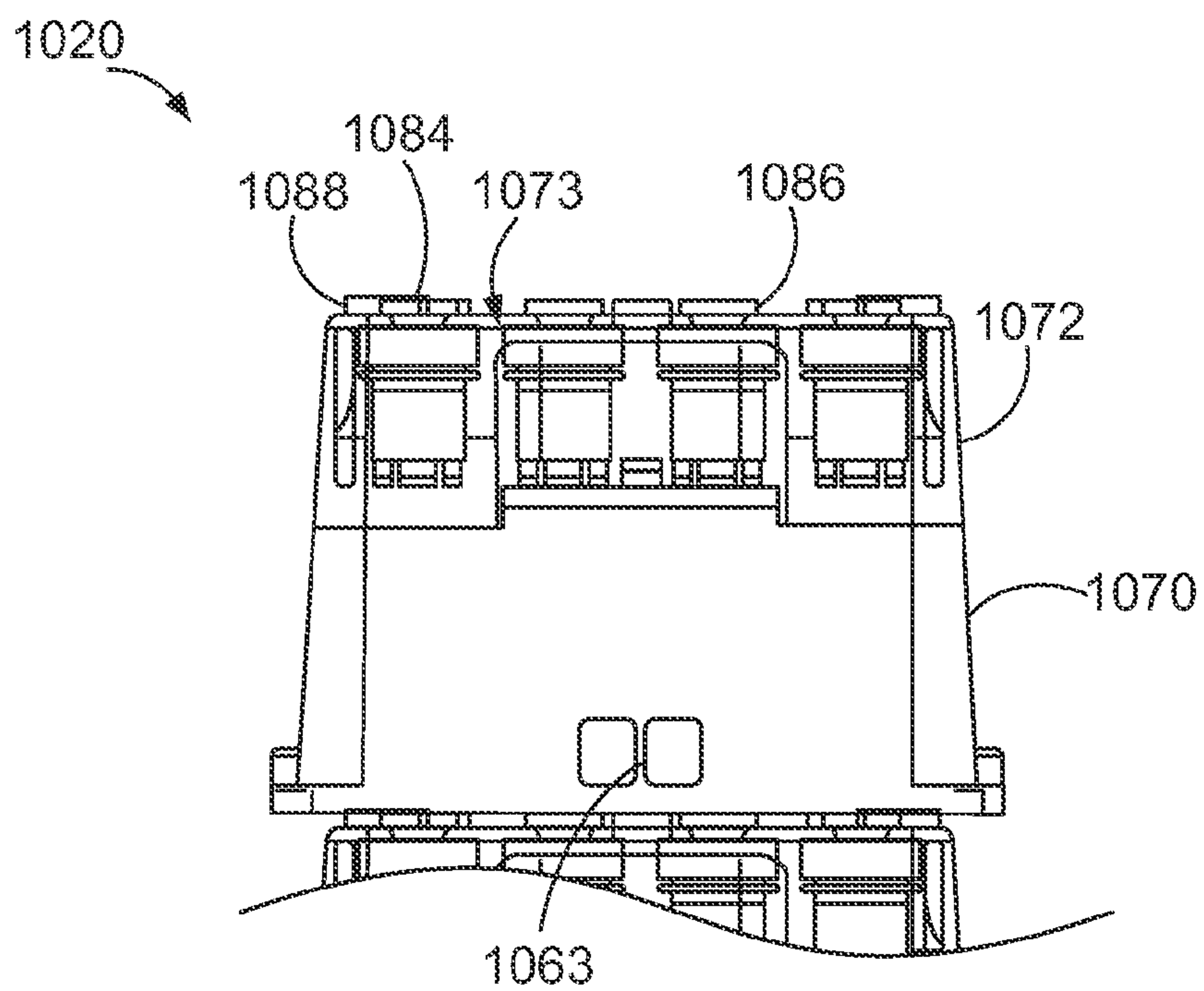


FIG. 34



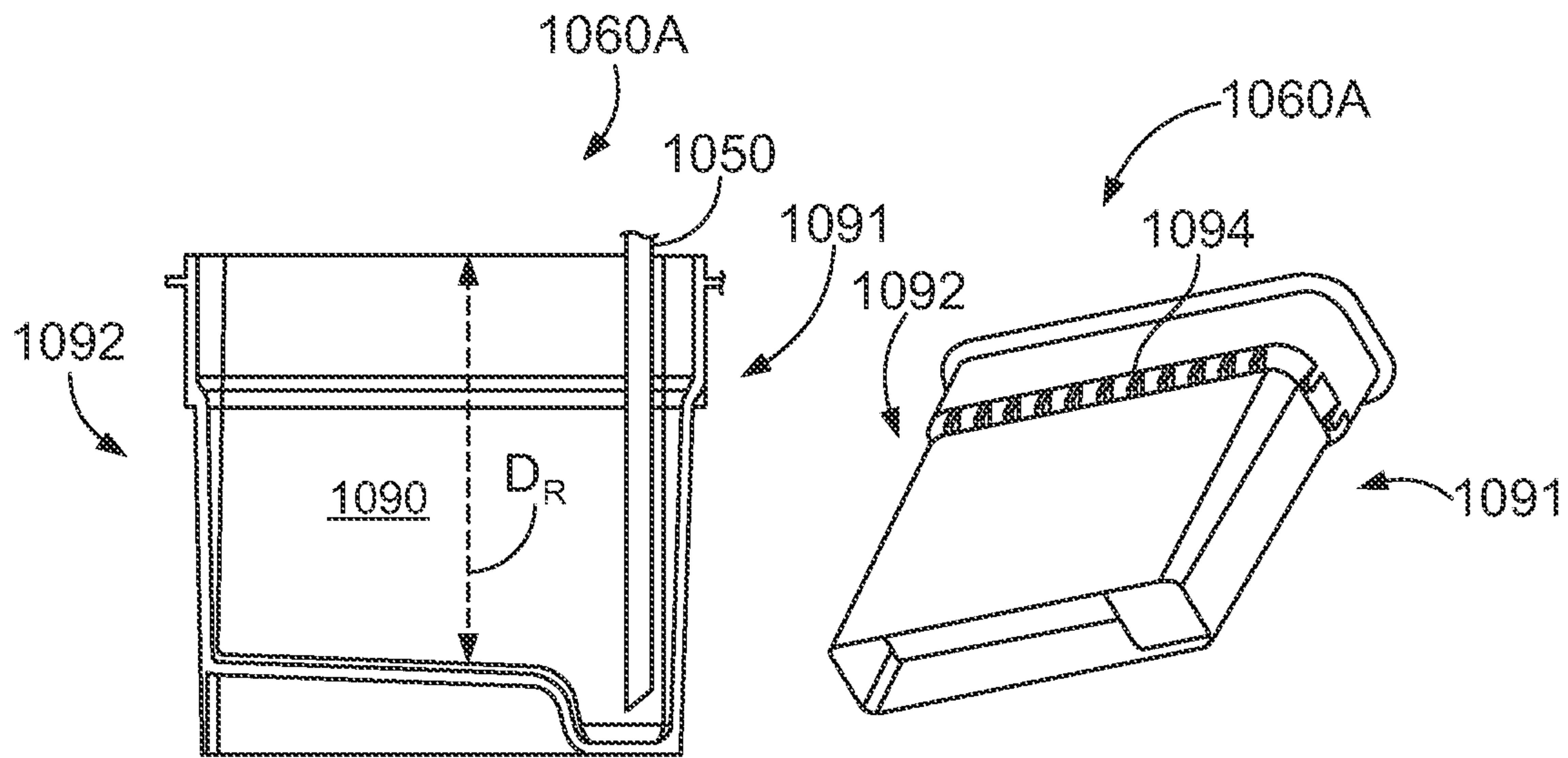


FIG. 36

FIG. 35

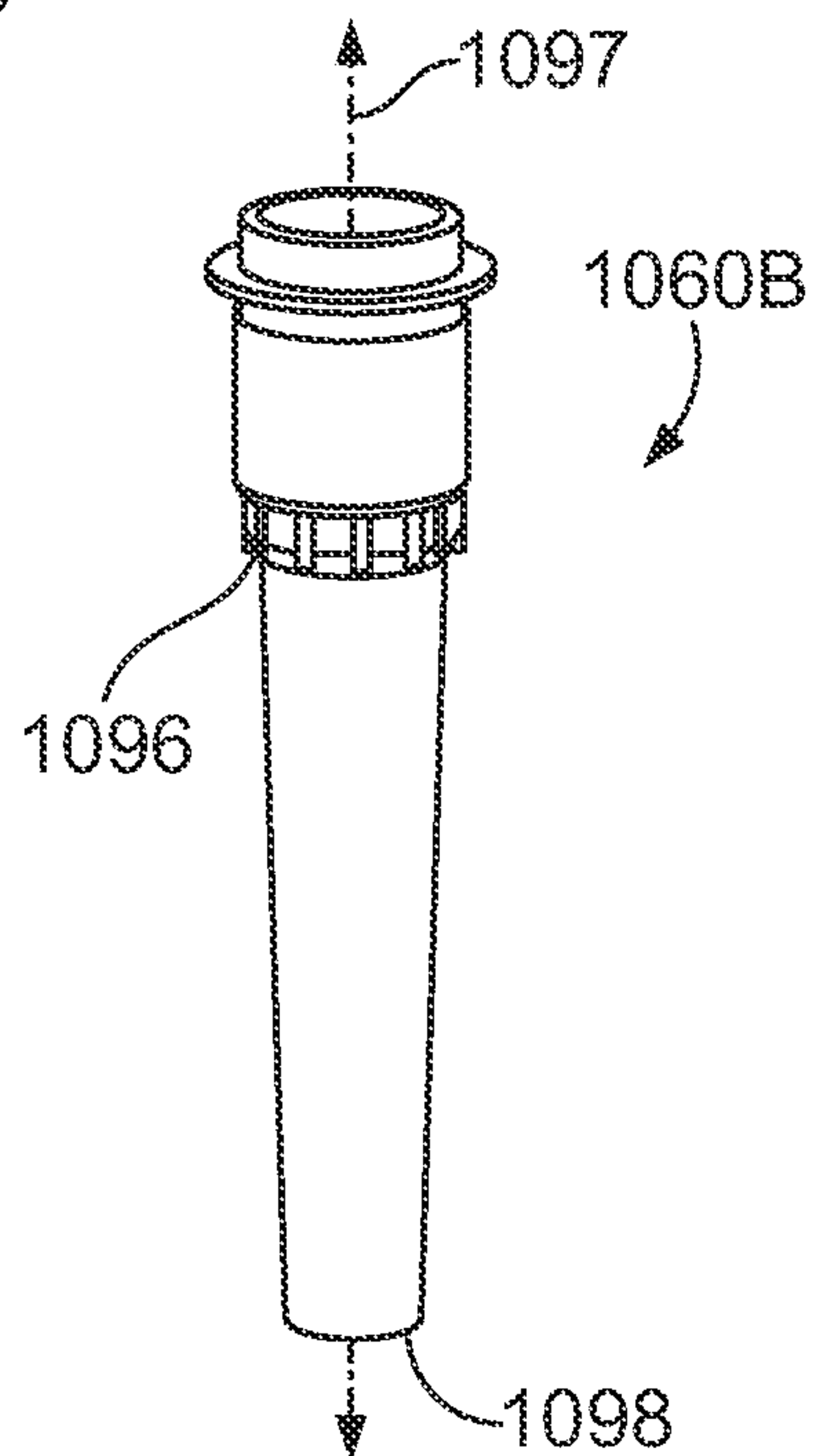


FIG. 37



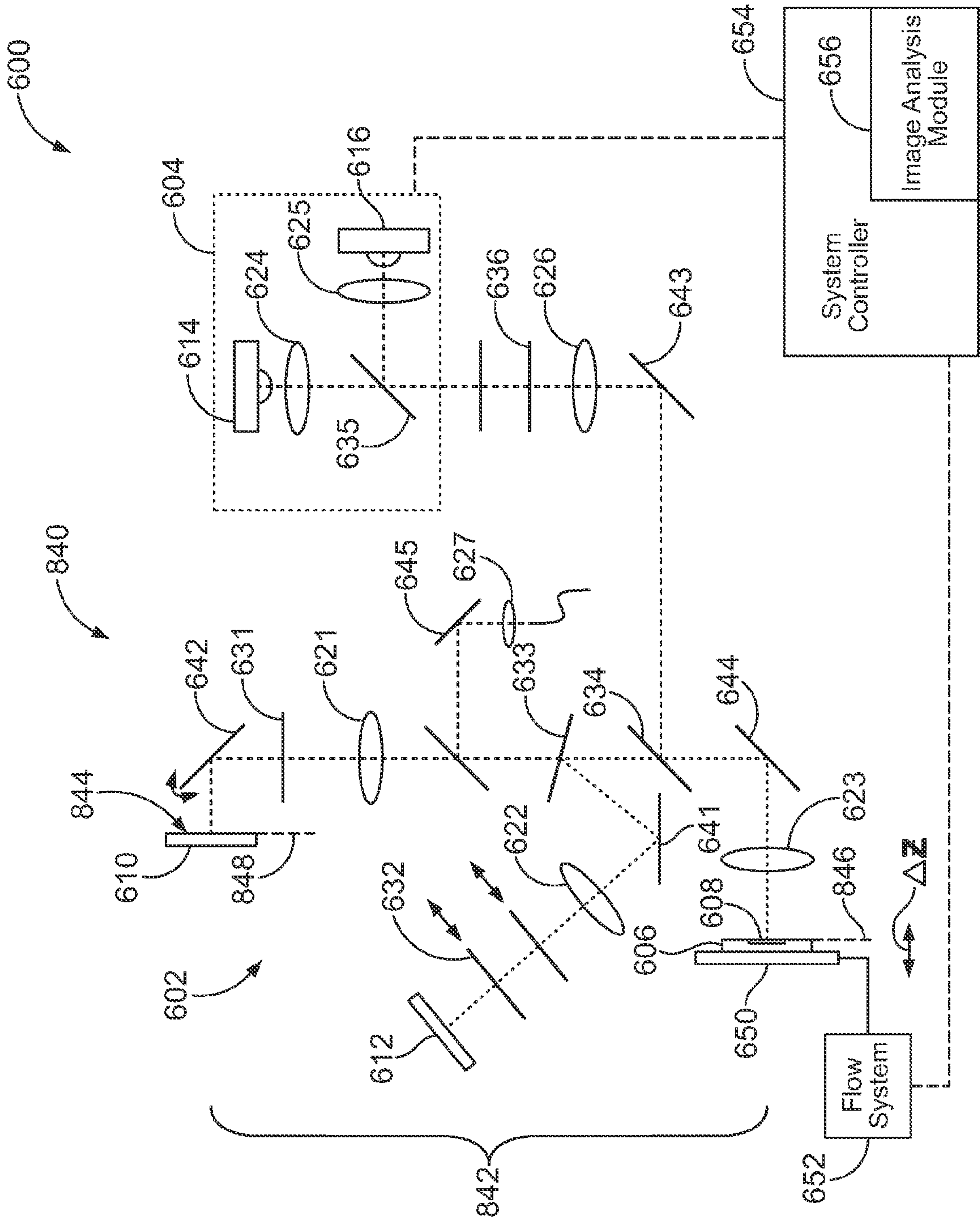


FIG. 38



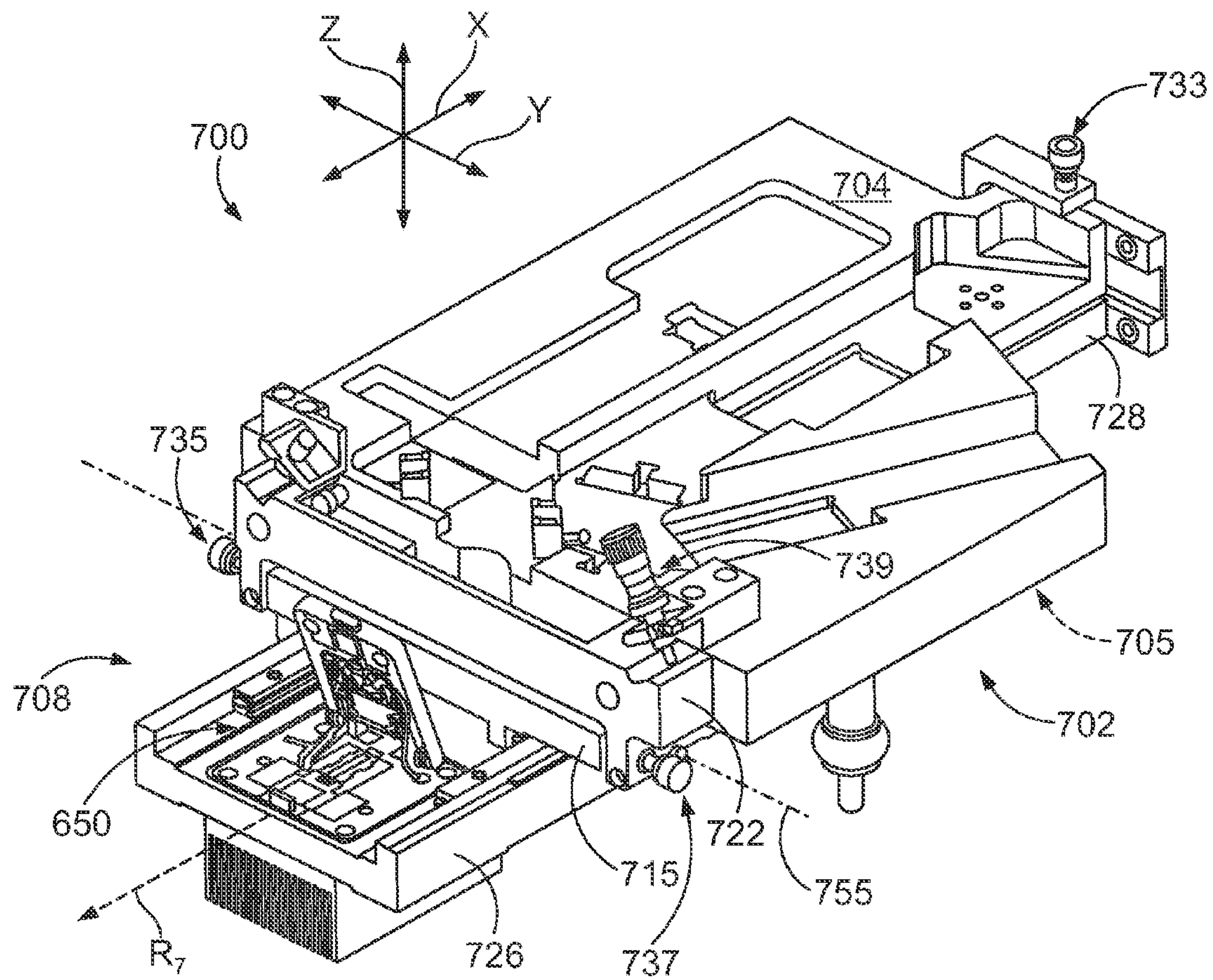


FIG. 39



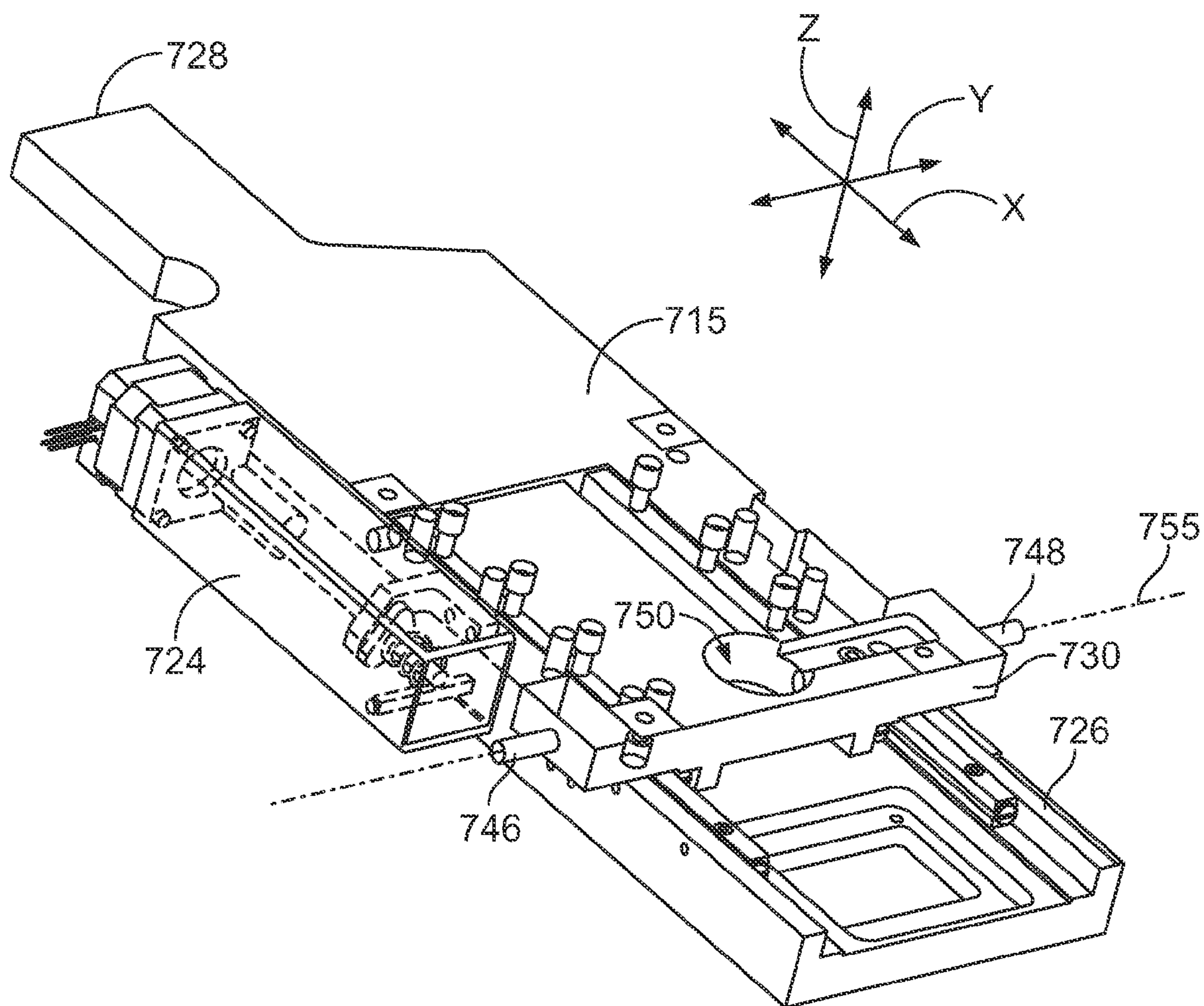


FIG. 40



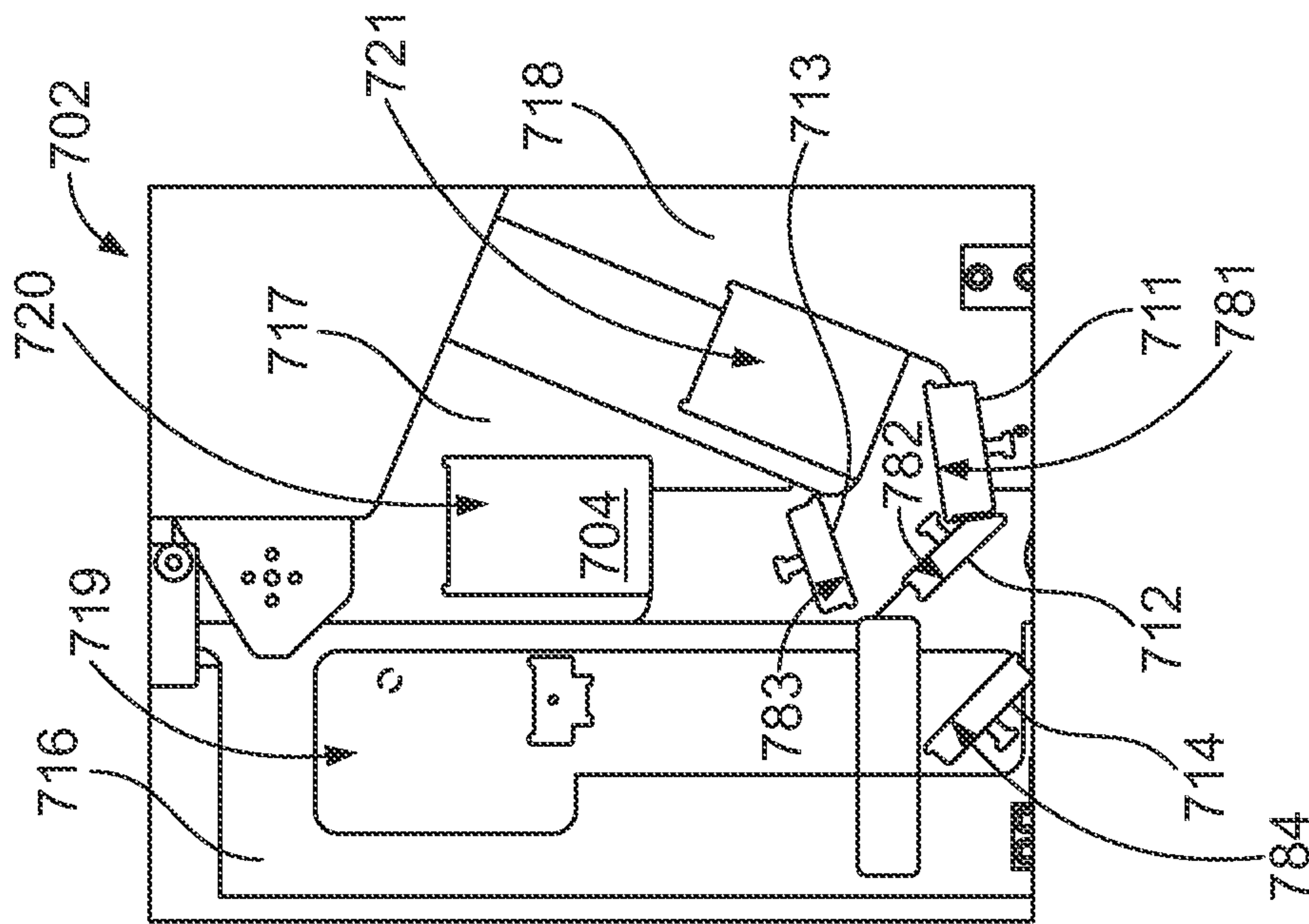


FIG. 42

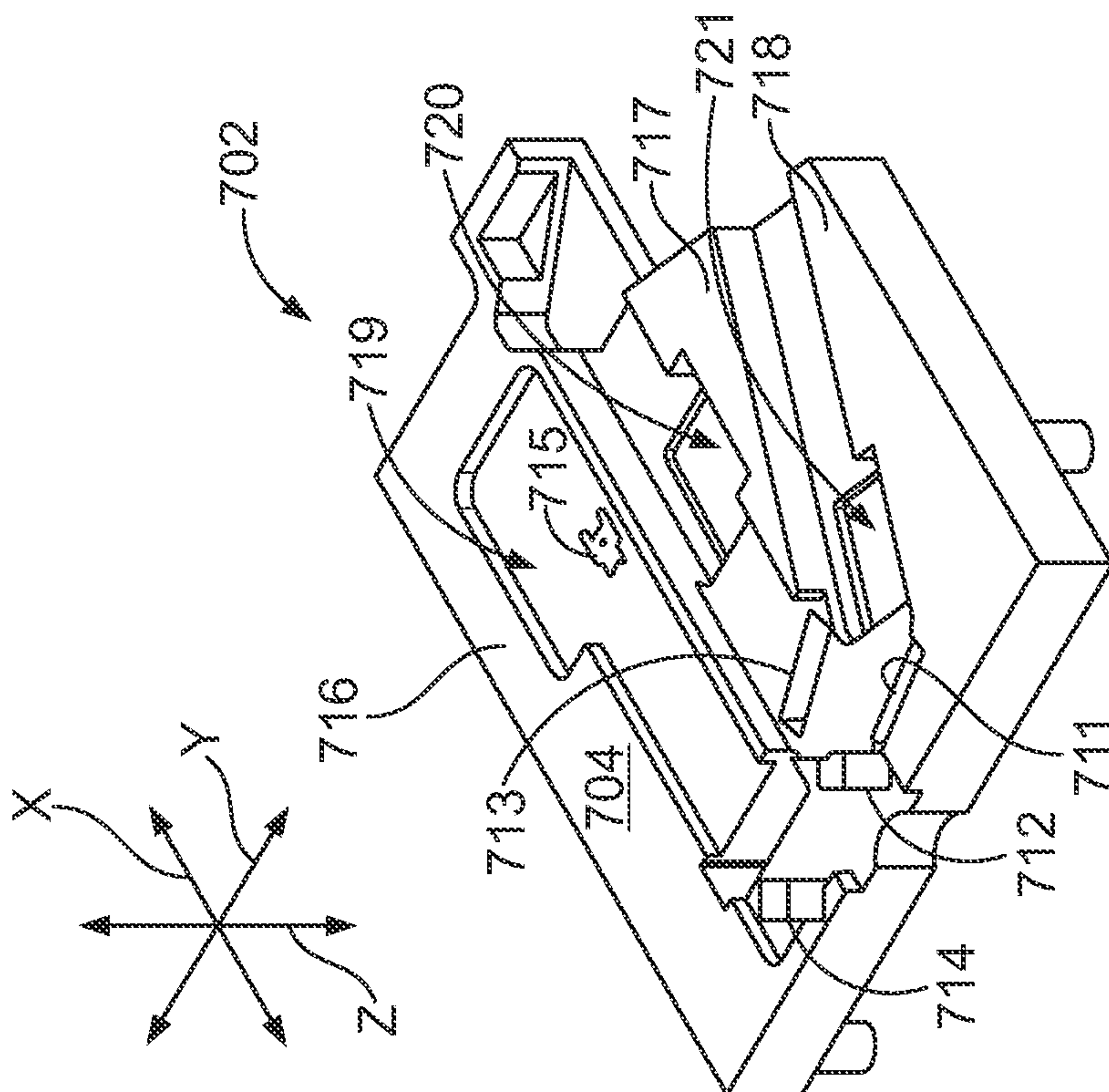
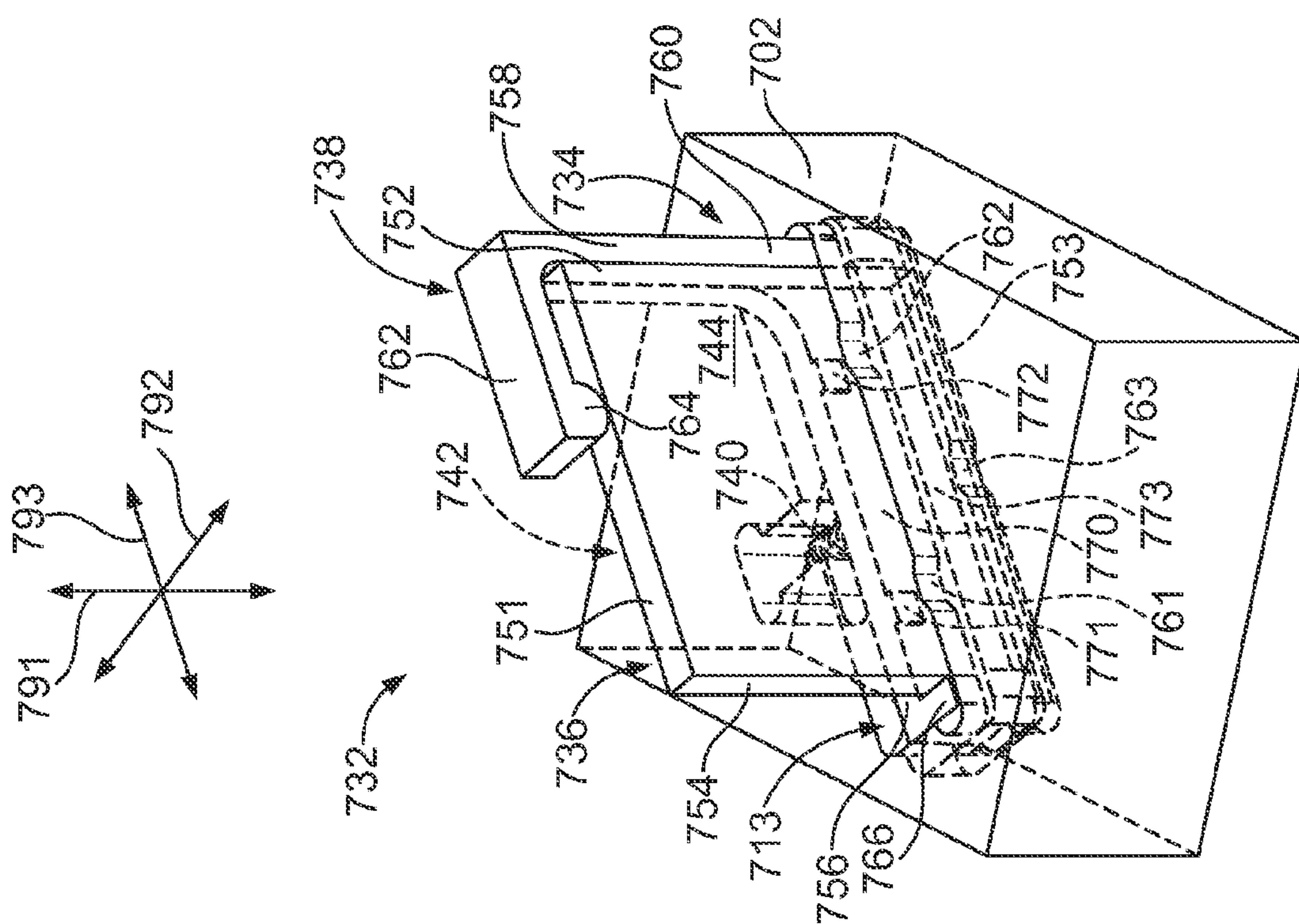
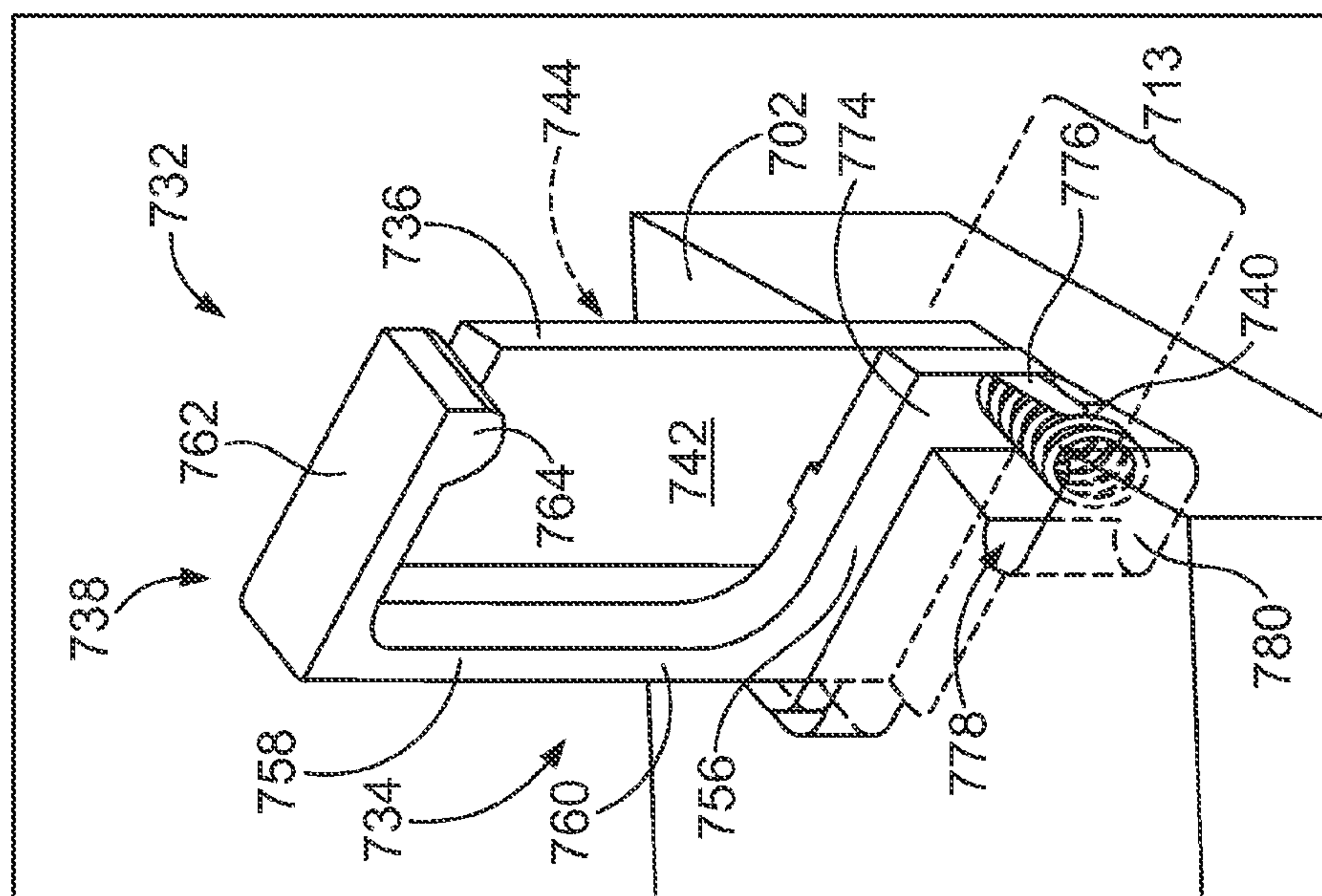


FIG. 41



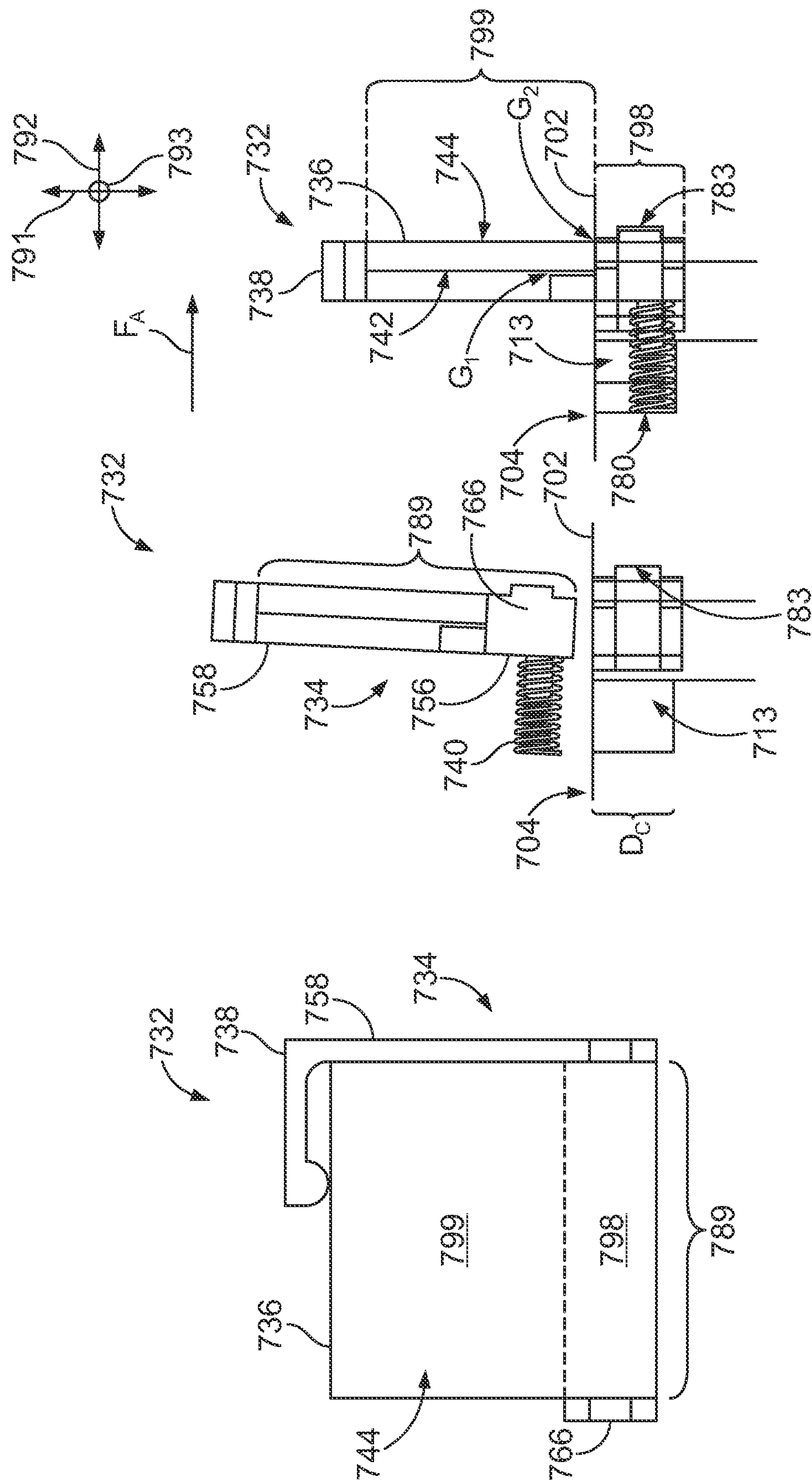



  
 DEPARTMENT OF HEALTH AND HUMAN SERVICES



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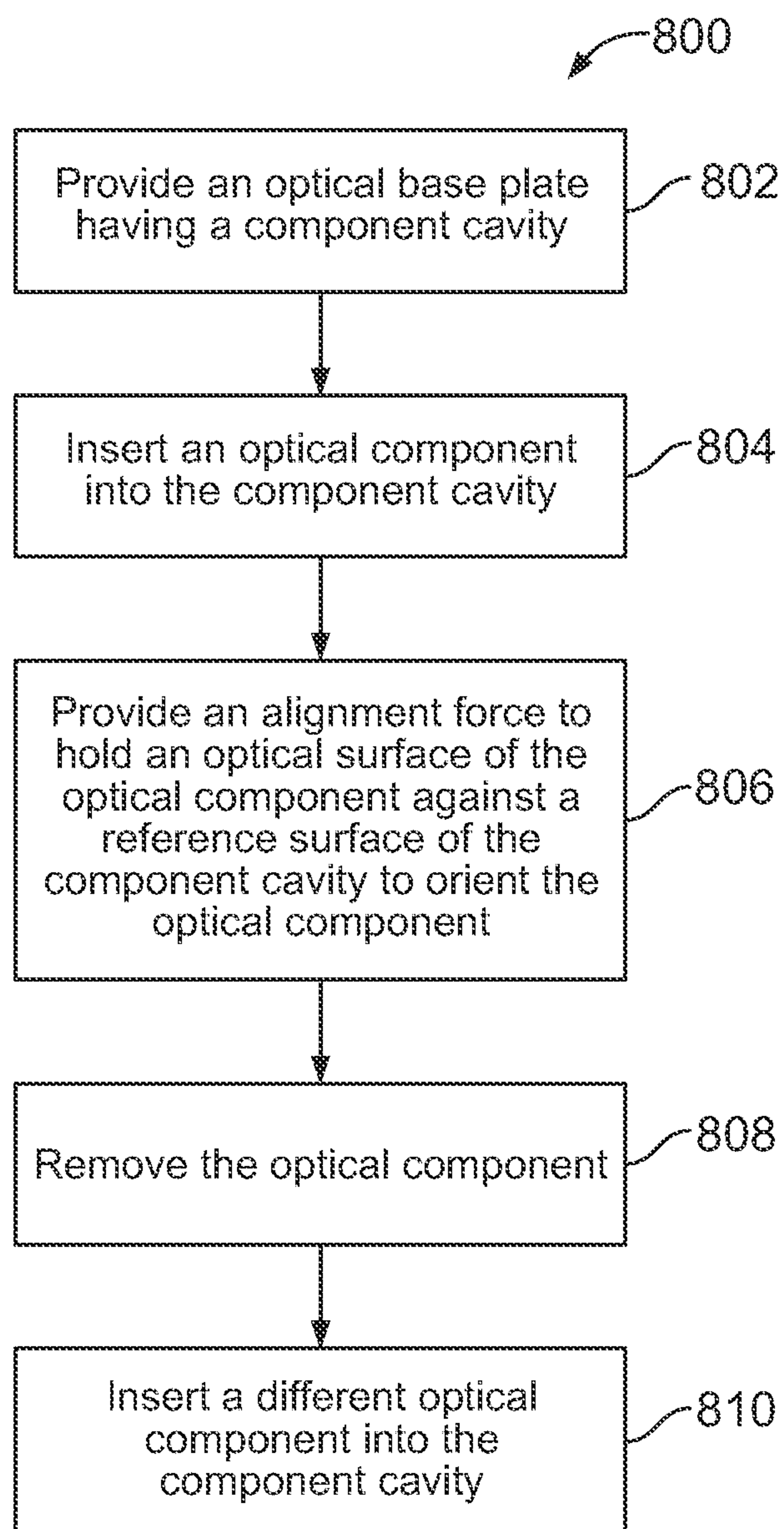


FIG. 47



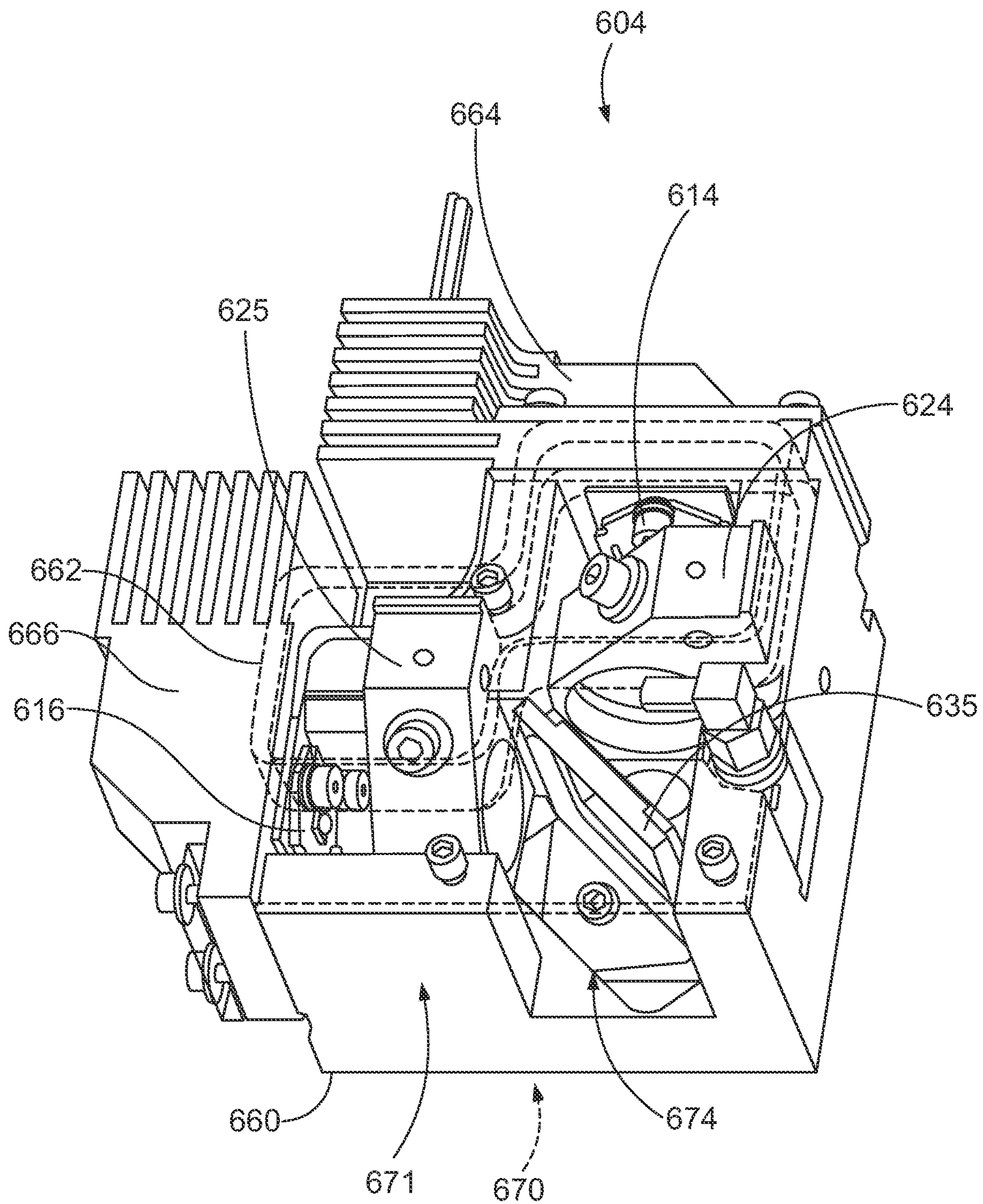


FIG. 48



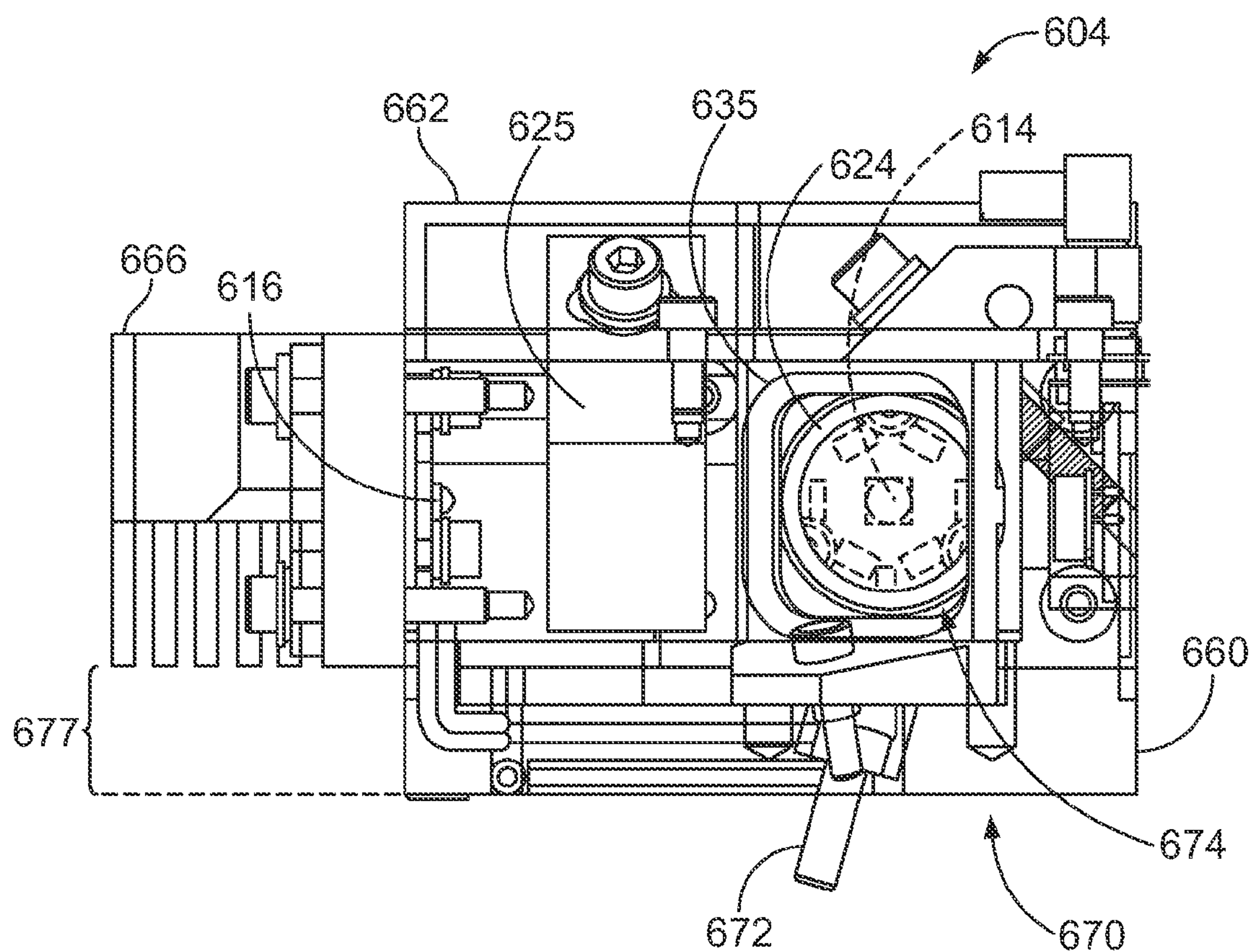


FIG. 49



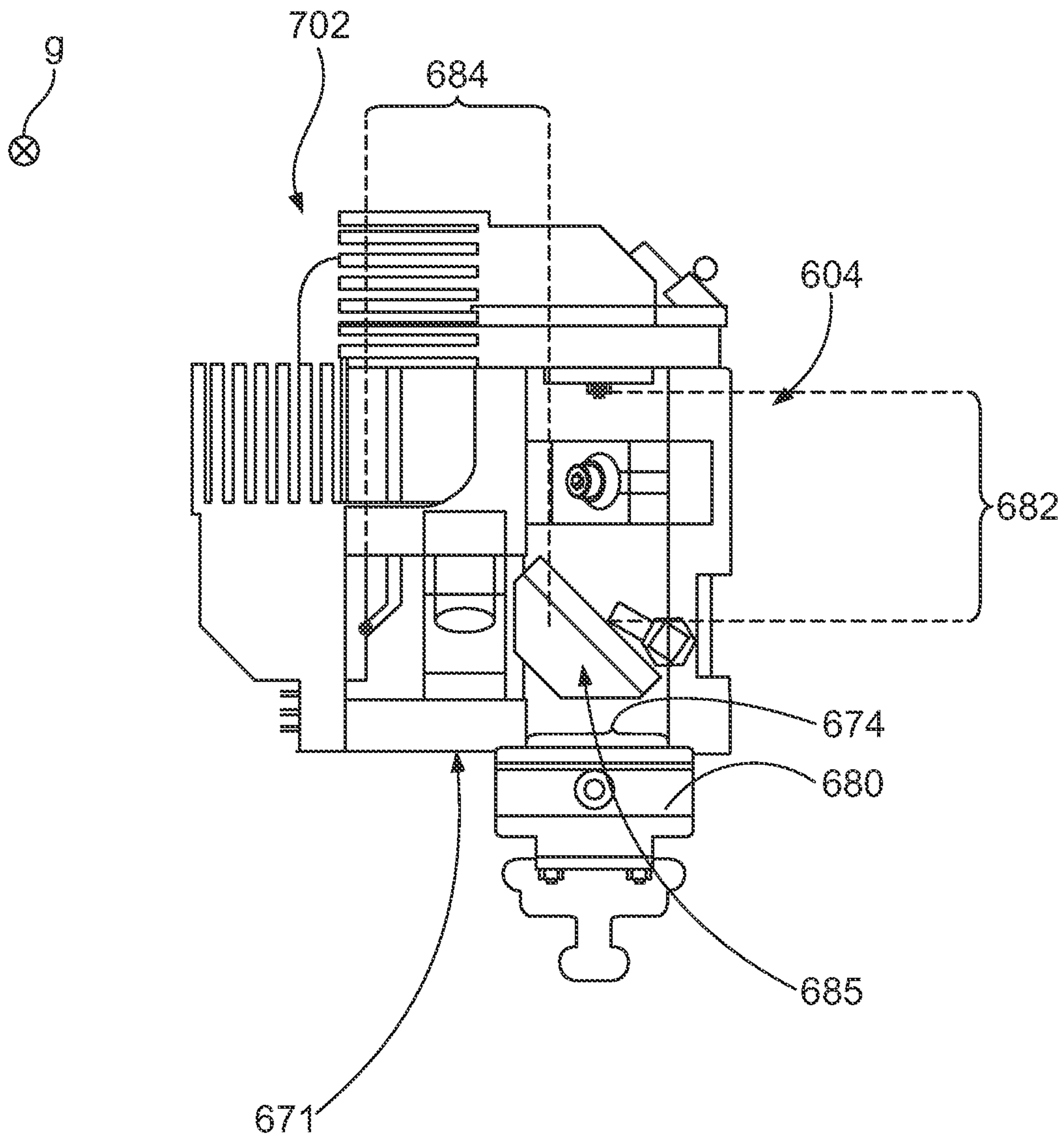


FIG. 50



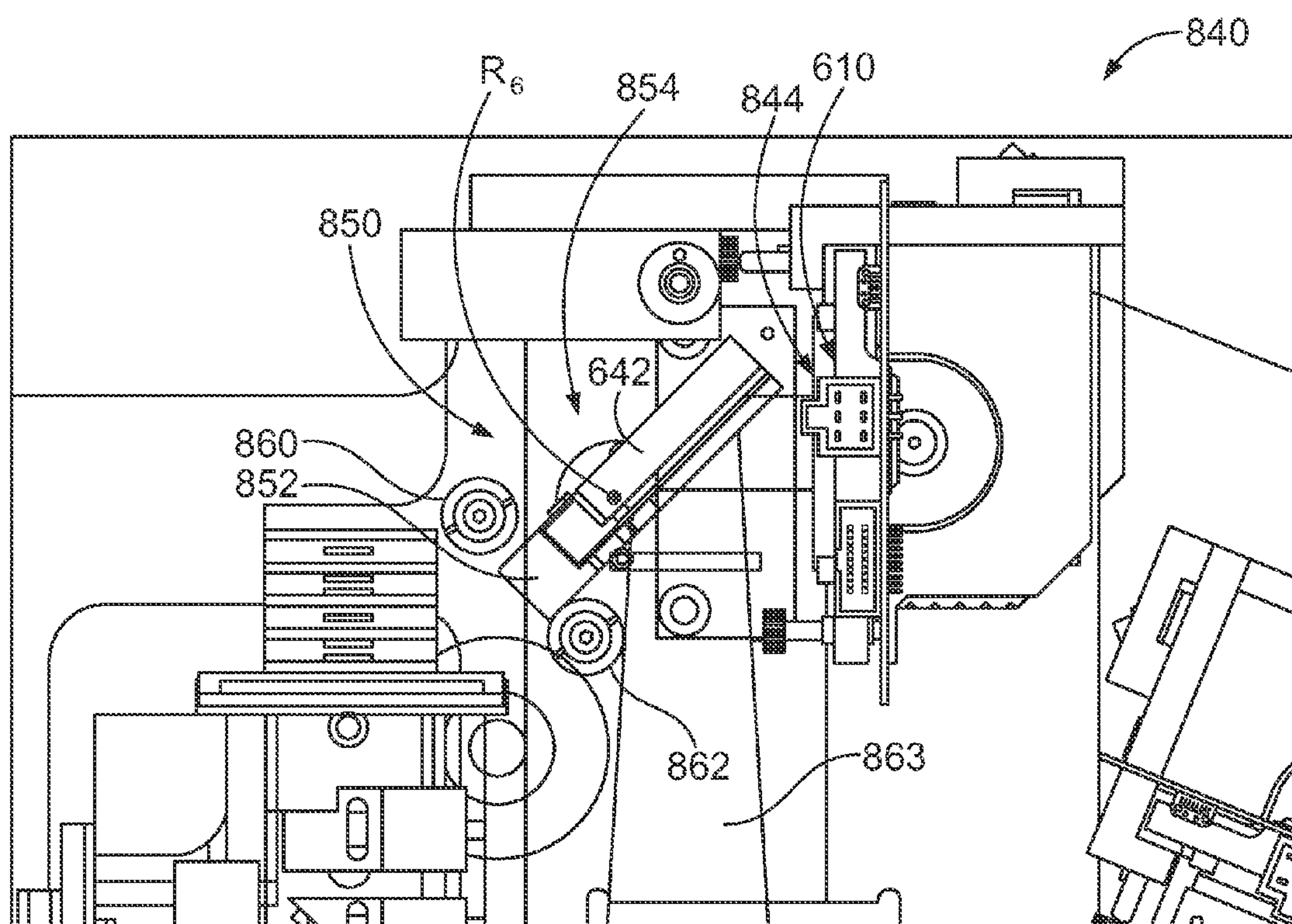


FIG. 51



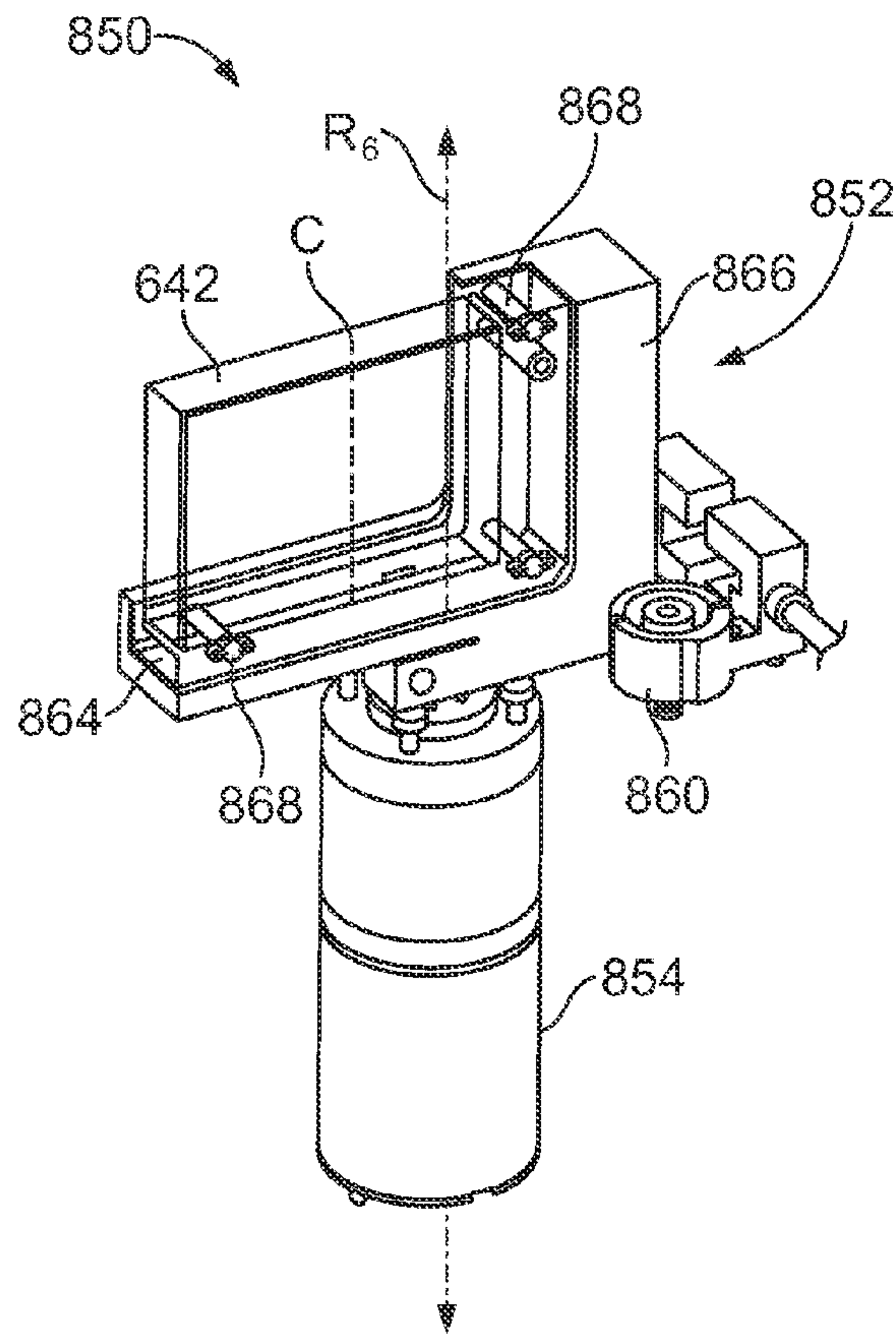


FIG. 52

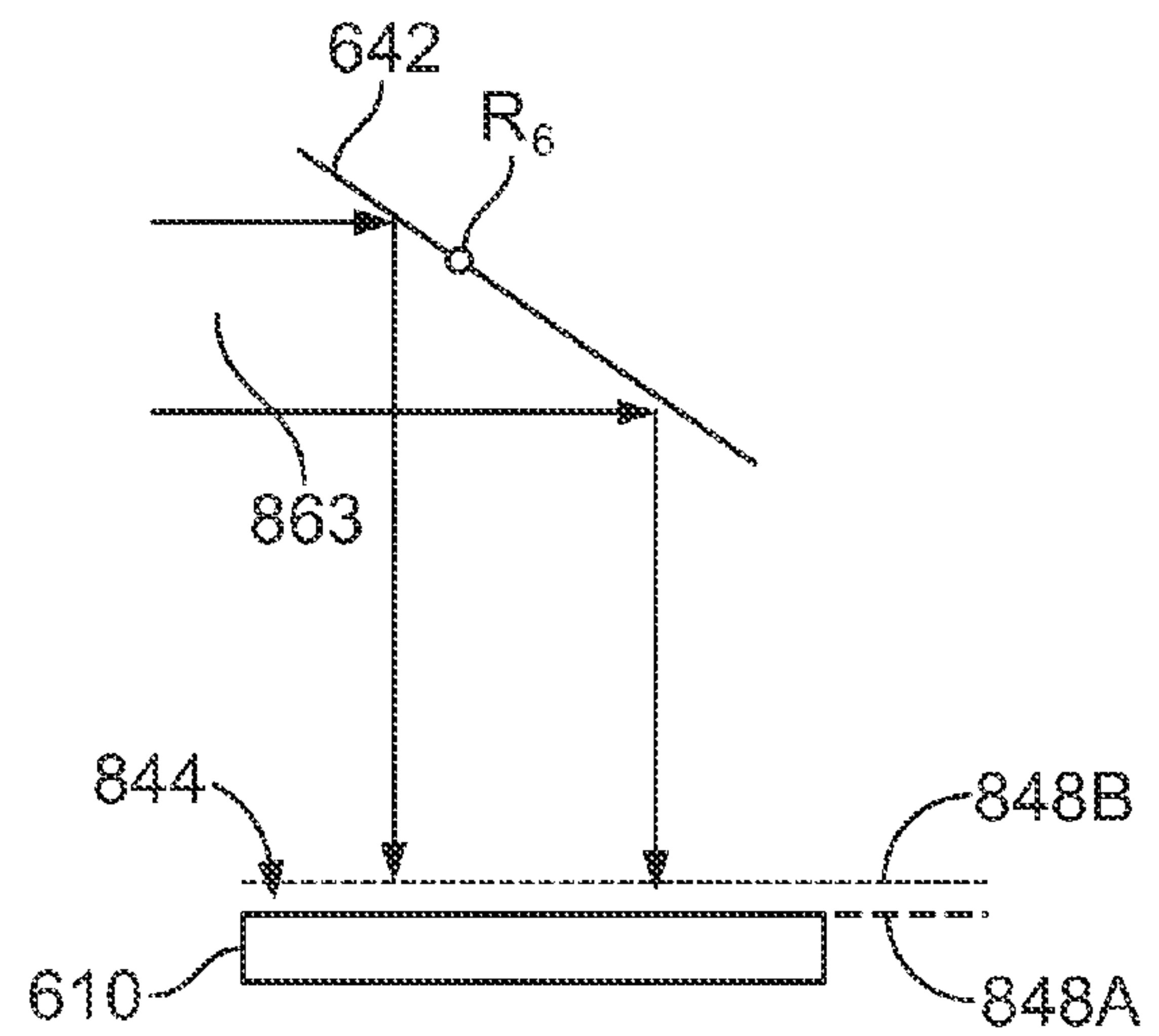


FIG. 53

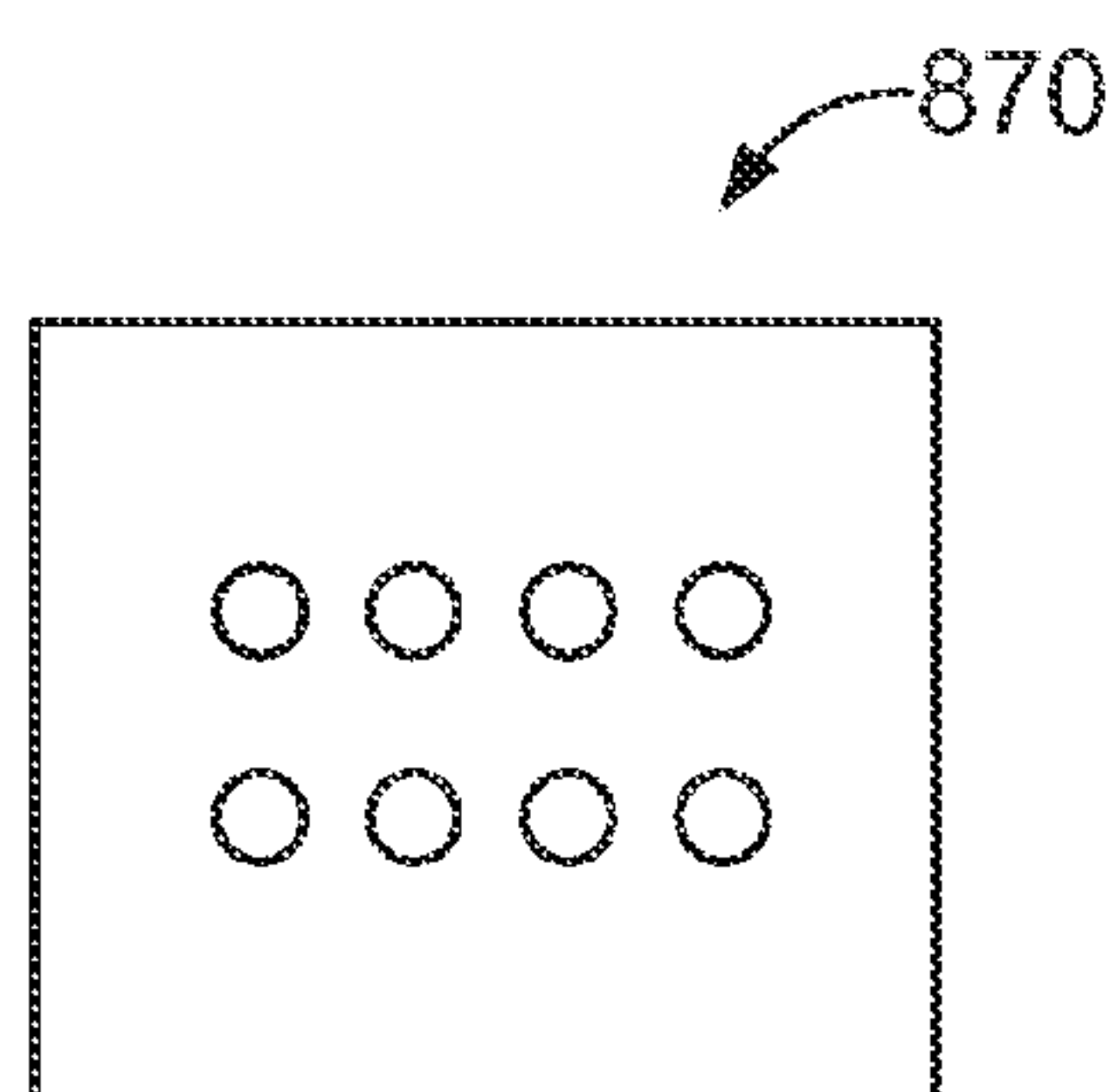


FIG. 54

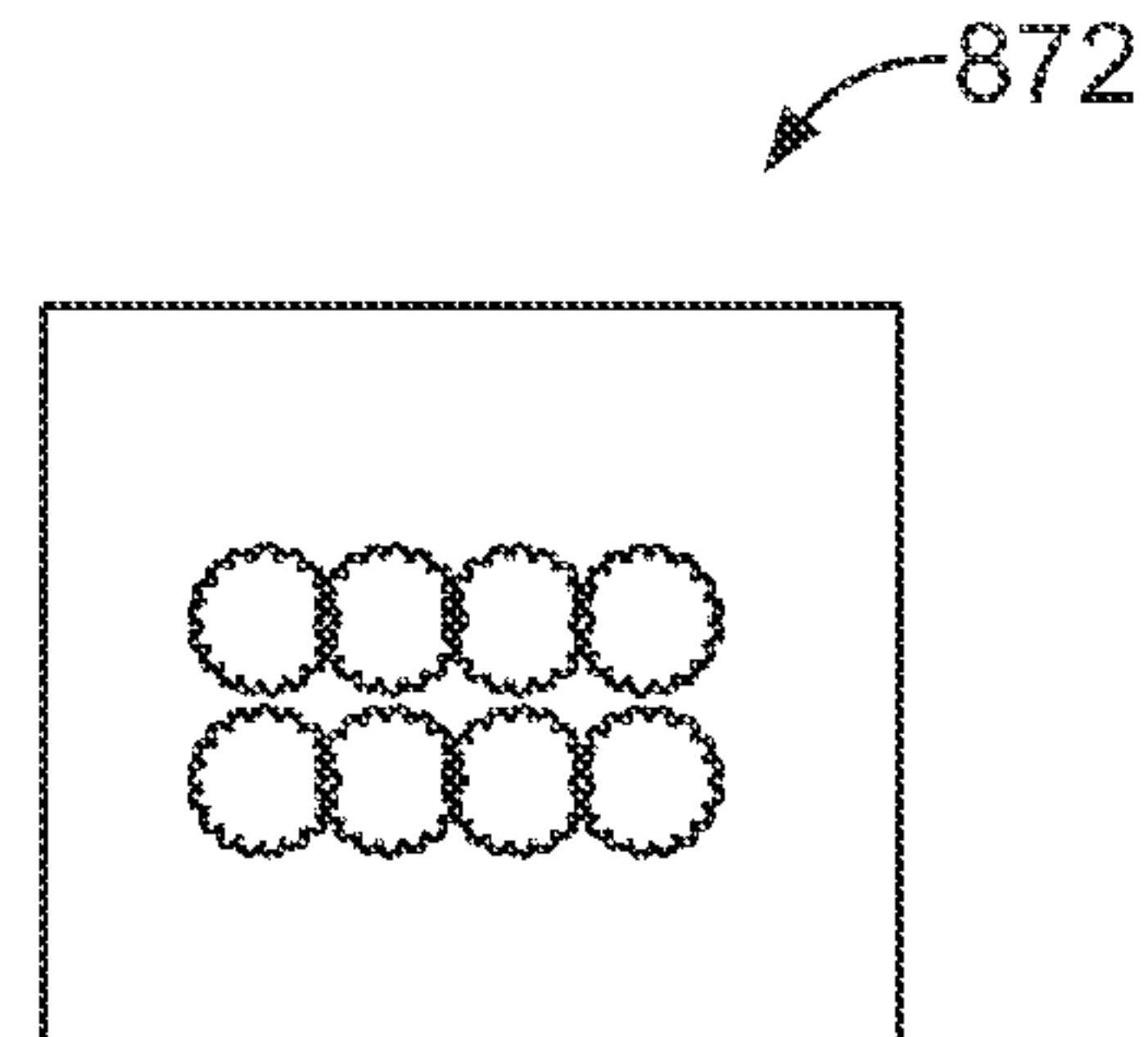


FIG. 55



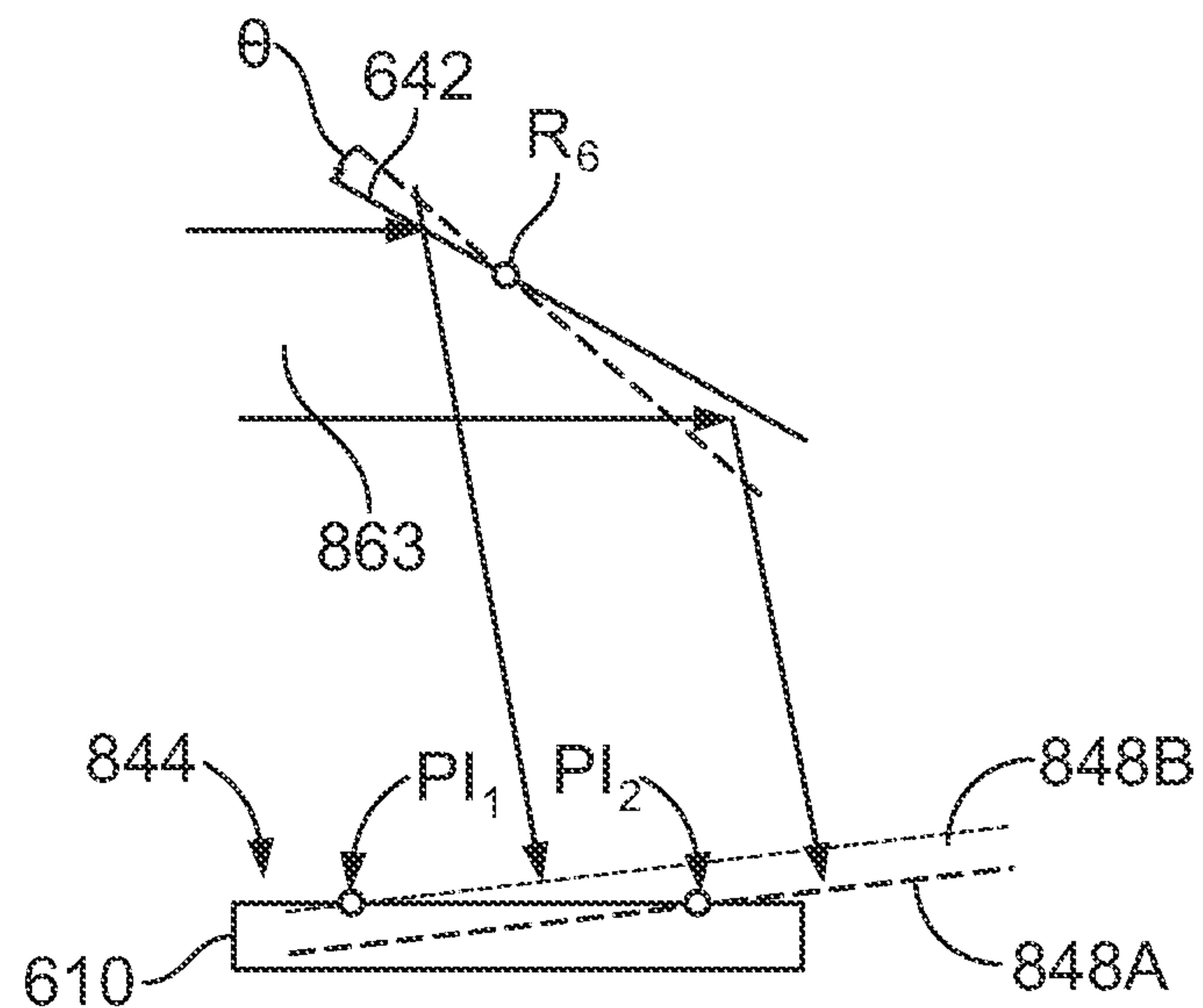


FIG. 56

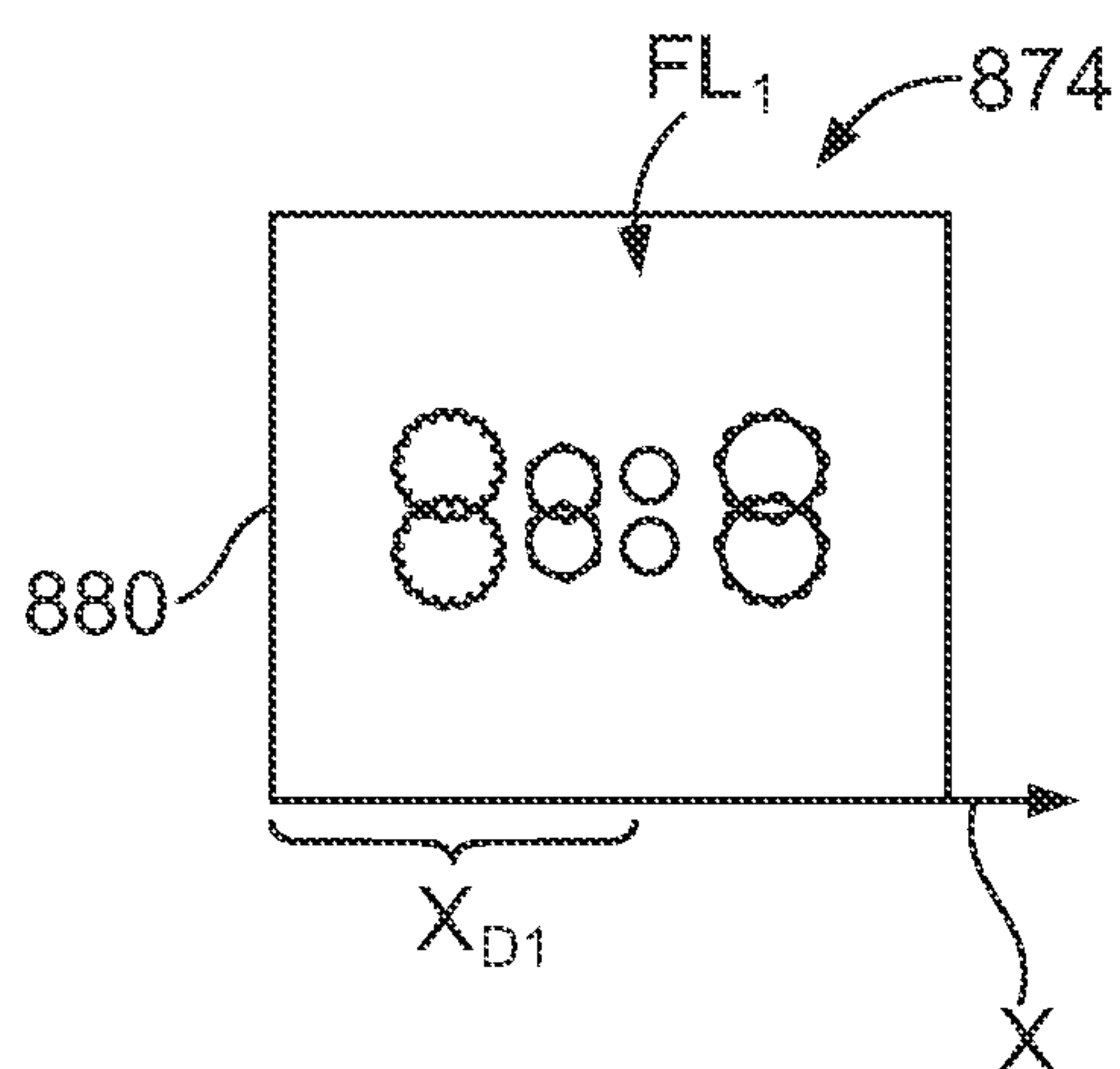


FIG. 57

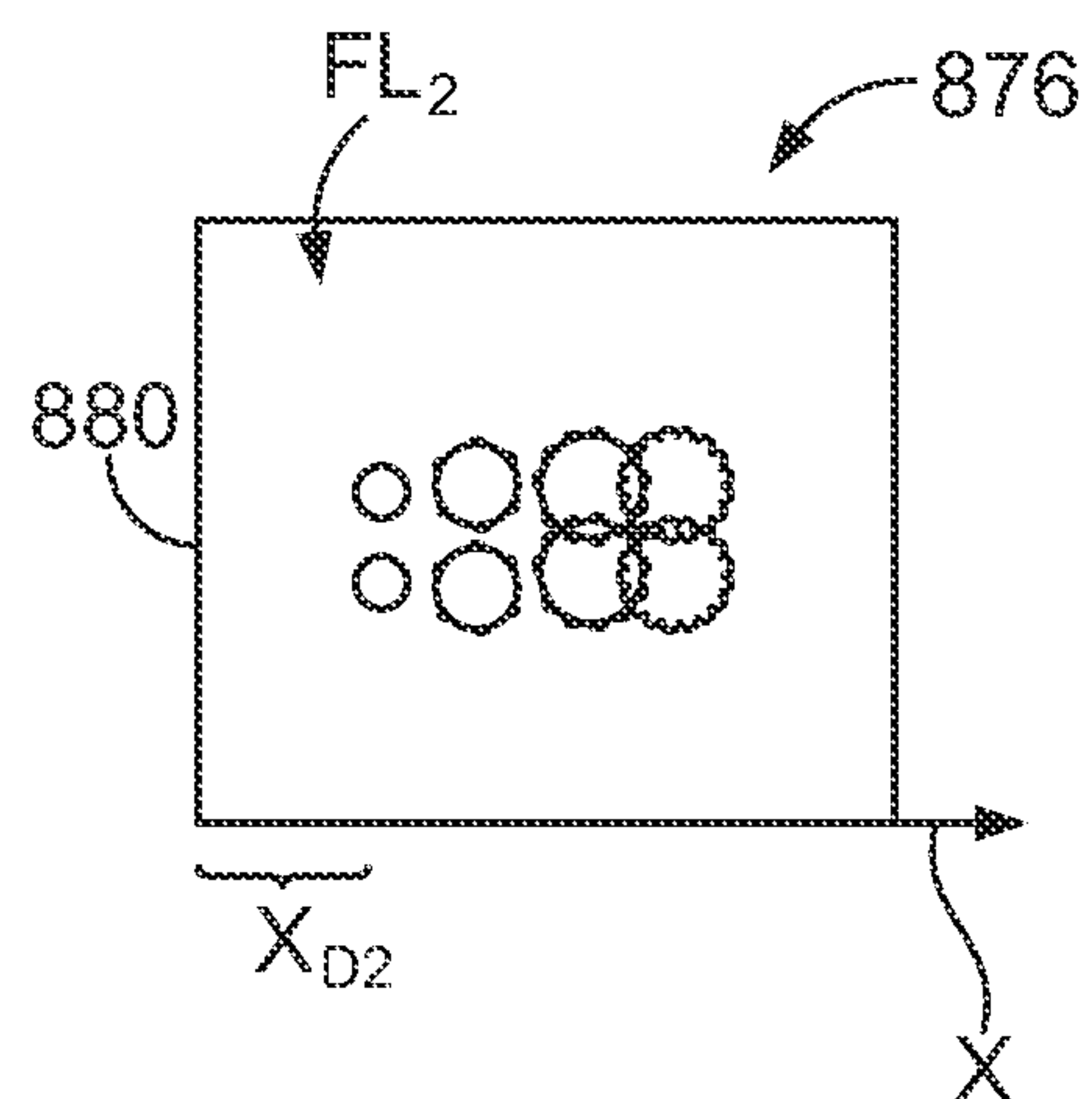


FIG. 58



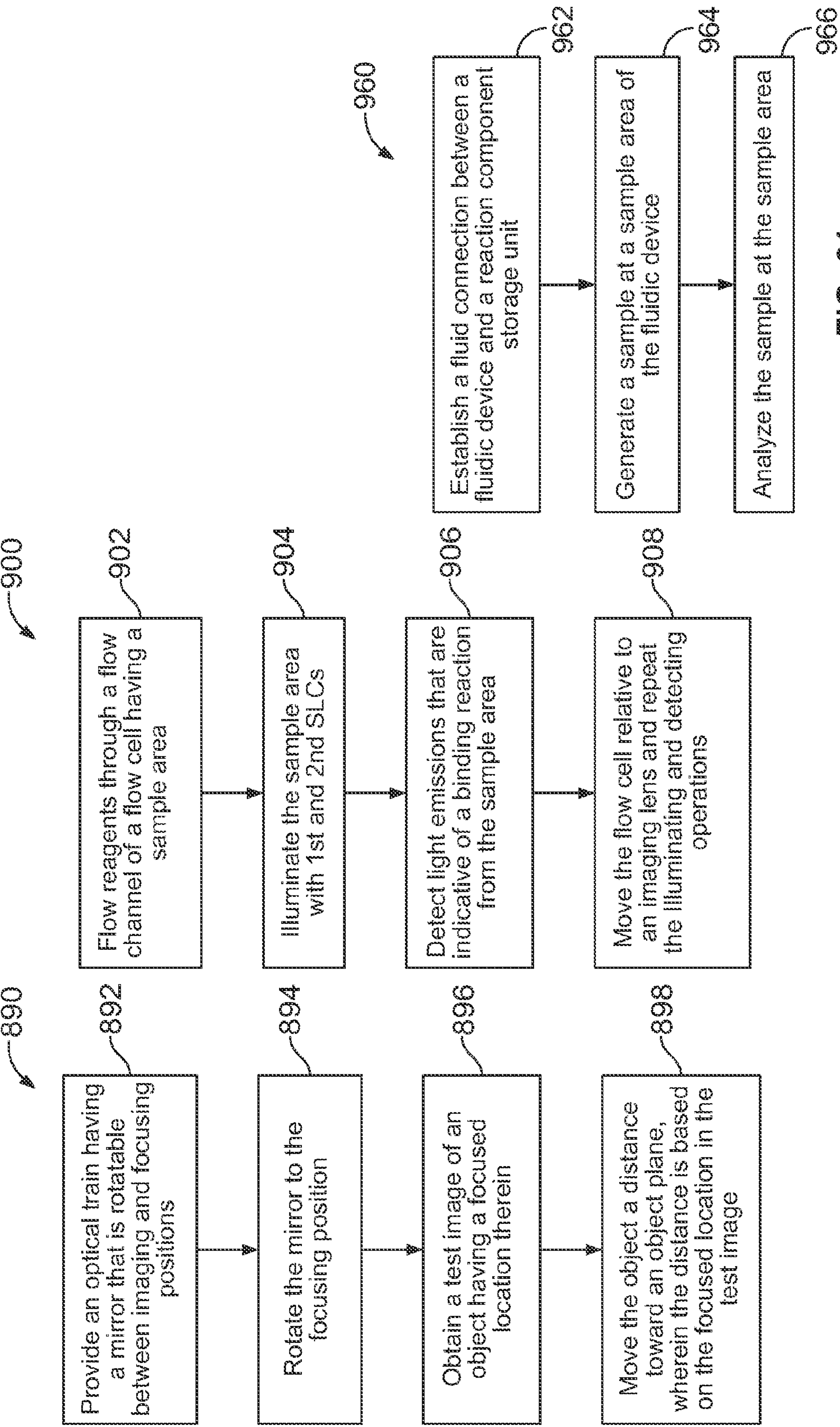


FIG. 59

FIG. 60

FIG. 61



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**SYSTEMS, METHODS, AND APPARATUSES  
TO IMAGE A SAMPLE FOR BIOLOGICAL  
OR CHEMICAL ANALYSIS****CROSS REFERENCE TO RELATED  
APPLICATIONS**

The present application relates to and claims the benefit of U.S. Provisional Application Ser. Nos. 61/431,425, filed on Jan. 10, 2011; 61/431,429, filed on Jan. 10, 2011; 61/431,439, filed on Jan. 11, 2011; 61/431,440, filed on Jan. 11, 2011; 61/438,486, filed on Feb. 1, 2011; 61/438,567, filed on Feb. 1, 2011; 61/438,530, filed on Feb. 1, 2011. Each of the above applications is herein incorporated by reference in its entirety.

**BACKGROUND OF THE INVENTION**

Embodiments of the present invention relate generally to biological or chemical analysis and more particularly, to assay systems having fluidic devices, optical assemblies, and/or other apparatuses that may be used in detecting desired reactions in a sample.

Various assay protocols used for biological or chemical research are concerned with performing a large number of controlled reactions. In some cases, the controlled reactions are performed on support surfaces. The desired reactions may then be observed and analyzed to help identify properties or characteristics of the chemicals involved in the desired reaction. For example, in some protocols, a chemical moiety that includes an identifiable label (e.g., fluorescent label) may selectively bind to another chemical moiety under controlled conditions. These chemical reactions may be observed by exciting the labels with radiation and detecting light emissions from the labels. The light emissions may also be provided through other means, such as chemiluminescence.

Examples of such protocols include DNA sequencing. In one sequencing-by-synthesis (SBS) protocol, clusters of clonal amplicons are formed through bridge PCR on a surface of a flow channel. After generating the clusters of clonal amplicons, the amplicons may be "linearized" to make single stranded DNA (sstDNA). A series of reagents is flowed into the flow cell to complete a cycle of sequencing. Each sequencing cycle extends the sstDNA by a single nucleotide (e.g., A, T, G, C) having a unique fluorescent label. Each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in one cycle. After nucleotides are added to the sstDNAs clusters, an image in four channels is taken (i.e., one for each fluorescent label). After imaging, the fluorescent label and the terminator are chemically cleaved from the sstDNA and the growing DNA strand is ready for another cycle. Several cycles of reagent delivery and optical detection can be repeated to determine the sequences of the clonal amplicons.

However, systems configured to perform such protocols may have limited capabilities and may not be cost-effective. Thus, there is a general need for improved systems, methods, and apparatuses that are capable of performing or being used during assay protocols, such as the SBS protocol described above, in a cost-effective, simpler, or otherwise improved manner.

**BRIEF DESCRIPTION OF THE INVENTION**

In accordance with one embodiment, a fluidic device for analyzing samples is provided. The fluidic device includes a flow cell having inlet and outlet ports and a flow channel extending therebetween. The flow cell is configured to hold a

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sample-of-interest. The fluidic device also includes a housing having a reception space that is configured to receive the flow cell. The reception space is sized and shaped to permit the flow cell to float relative to the housing. The fluidic device also includes a gasket that is coupled to the housing. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively.

In another embodiment, a removable cartridge configured to hold and facilitate positioning a flow cell for imaging is provided. The cartridge includes a removable housing that has a reception space configured to hold the flow cell substantially within an object plane. The housing includes a pair of housing sides that face in opposite directions. The reception space extends along at least one of the housing sides so that the flow cell is exposed to an exterior of the housing through said at least one of the housing sides. The cartridge also includes a cover member that is coupled to the housing and includes a gasket. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is configured to be mounted over an exposed portion of the flow cell when the flow cell is held by the housing.

In yet another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes positioning a removable fluidic device on a support surface of an imaging system. The device has a reception space, a flow cell located within the reception space, and a gasket. The flow cell extends along an object plane in the reception space and is floatable relative to the gasket within the object plane. The method also includes moving the flow cell within the reception space while on the support surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket.

In another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes providing a fluidic device having a housing that includes a reception space and a floatable flow cell located within the reception space. The housing has recesses that are located immediately adjacent to the reception space. The method also includes positioning the fluidic device on a support structure having alignment members. The alignment members are inserted through corresponding recesses. The method also includes moving the flow cell within the reception space. The alignment members engage edges of the flow cell when the flow cell is moved within the reception space.

In another embodiment, a fluidic device holder is provided that is configured to orient a sample area with respect to mutually perpendicular X, Y, and Z-axes. The device holder includes a support structure that is configured to receive a fluidic device. The support structure includes a base surface that faces in a direction along the Z-axis and is configured to have the device positioned thereon. The device holder also includes a plurality of reference surfaces in respective directions along an XY-plane and an alignment assembly that includes an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end toward and away from the reference surfaces. The locator arm is configured to hold the device against the reference surfaces when the locator arm is in the biased position.

In another embodiment, a fluidic device holder is provided that includes a support structure having a loading region for receiving a fluidic device. The support structure includes a base surface that partially defines the loading region and is



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configured to have the device positioned thereon. The device holder includes a cover assembly that is coupled to the support structure and is configured to be removably mounted over the device. The cover assembly includes a cover housing having housing legs and a bridge portion that joins the housing legs. The housing legs extend in a common direction and have a viewing space that is located therebetween. The viewing space is positioned above the loading region.

In another embodiment, a method for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes is provided. The method includes providing an alignment assembly that has a movable locator arm having an engagement end. The locator arm is movable between retracted and biased positions. The method also includes positioning a fluidic device on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. The device has a sample area. The method also includes moving the locator arm to the biased position. The locator arm presses the device against the reference surfaces such that the device is held in a fixed position.

In yet another embodiment, an optical assembly is provided that includes a base plate having a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The optical assembly also includes an optical component having an optical surface that is configured to reflect light or transmit light therethrough. The optical assembly also includes a mounting device that has a component retainer and a biasing element that is operatively coupled to the retainer. The retainer holds the optical component so that a space portion of the optical surface faces the reference surface and a path portion of the optical surface extends beyond the support side into an optical path. The biasing element provides an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, a method of assembling an optical train is provided. The method includes providing a base plate that has a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The method also includes inserting an optical component into the component-receiving space. The optical component has an optical surface that is configured to reflect light or transmit light therethrough. The optical surface has a space portion that faces the reference surface and a path portion that extends beyond the support side into an optical path. The method also includes providing an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, an optical imaging system is provided that includes an object holder to hold and move an object and a detector to detect optical signals from the object at a detector surface. The imaging system also includes an optical train that is configured to direct the optical signals onto the detector surface. The optical train has an object plane that is proximate to the object holder and an image plane that is proximate to the detector surface. The optical train includes

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a mirror that is rotatable between an imaging position and a focusing position. The imaging system also includes an image analysis module that is configured to analyze a test image detected at the detector surface when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location in the test image is indicative of a position of the object with respect to the object plane. The object holder is configured to move the object toward the object plane based on the focused location.

In another embodiment, a method for controlling focus of an optical imaging system is provided. The method includes providing an optical train that is configured to direct optical signals onto a detector surface. The optical train has an object plane that is proximate to an object and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The method also includes rotating the mirror to the focusing position and obtaining a test image of the object when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location is indicative of a position of the object with respect to the object plane. The method also includes moving the object toward the object plane based on the focused location.

In another embodiment, an optical imaging system is provided that includes a sample holder configured to hold a flow cell. The flow cell includes a flow channel having a sample area. The imaging system also includes a flow system that is coupled to the flow cell and configured to direct reagents through the flow channel to the sample area. The imaging system also includes an optical train that is configured to direct excitation light onto the sample area and first and second light sources. The first and second light sources have fixed positions with respect to the optical train. The first and second light sources provide first and second optical signals, respectively, for exciting the biomolecules. The imaging system also includes a system controller that is communicatively coupled to the first and second light sources and to the flow system. The controller is configured to activate the flow system to flow the reagents to the sample area and activate the first and second light sources after a predetermined synthesis time period. The light sources can be, for example, lasers or semiconductor light sources (SLSSs), such as laser diodes or light emitting diodes (LEDs).

In another embodiment, a method of performing a biological assay is provided. The method includes flowing reagents through a flow channel having a sample area. The sample area includes biomolecules that are configured to chemically react with the reagents. The method also includes illuminating the sample area with first and second light sources. The first and second light sources provide first and second optical signals, respectively. The biomolecules provide light emissions indicative of a binding reaction when illuminated by the first or second light sources. The method also includes detecting the light emissions from the sample area. The light sources can be, for example, lasers or semiconductor light sources (SLSSs), such as a laser diodes or light emitting diodes (LEDs).

In another embodiment, a flow cell is provided that includes a first layer that has a mounting surface and an outer surface that face in opposite directions and that define a thickness therebetween. The flow cell also includes a second layer having a channel surface and an outer surface that face in opposite directions and that define a thickness therebetween. The second layer has a grooved portion that extends along the channel surface. The channel surface of the second layer is secured to the mounting surface. The flow cell also



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includes a flow channel that is defined by the grooved portion of the channel surface and a planar section of the mounting surface. The flow channel includes an imaging portion. The thickness of the second layer is substantially uniform along the imaging portion and is configured to transmit optical signals therethrough. The thickness of the first layer is substantially uniform along the imaging portion and is configured to permit uniform transfer of thermal energy there-through.

In another embodiment, a light source module is provided that includes a module frame having a light passage and a light source that is secured to the module frame and oriented to direct optical signals through the light passage along an optical path. The light source module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the light source. The optical component is located within the light passage such that the optical component is within the optical path.

In another embodiment, an excitation light module is provided that includes a module frame and first and second semiconductor light sources (SLSs) that are secured to the module frame. The first and second SLSs have fixed positions with respect to each other. The first and second SLSs are configured to provide different excitation optical signals. The excitation light module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the first and second SLSs. The optical component permits the optical signals from the first SLS to transmit therethrough and reflects the optical signals from the second SLS. The reflected and transmitted optical signals are directed along a common path out of the module frame.

In one embodiment, a method of performing a biological or chemical assay is provided. The method includes establishing a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays. The reaction components include sample-generation components and sample-analysis components. The method also includes generating a sample at the sample area of the fluidic device. The generating operation includes flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. The method also includes analyzing the sample at the sample area. The analyzing operation includes flowing at least one sample-analysis component to the sample area. Said at least one sample-analysis component reacts with the sample to provide optically detectable signals indicative of an event-of-interest. The generating and analyzing operations are conducted in an automated manner by the assay system.

In another embodiment, an assay system is provided that includes a fluidic device holder that is configured to hold a fluidic device and establish a fluid connection with the fluidic device. The assay system also includes a fluidic network that is configured to fluidically connect the fluidic device to a reaction component storage unit. The assay system also includes a fluidic control system that is configured to selectively flow fluids from the storage unit through the fluidic device. Furthermore, the assay system includes a system controller that has a fluidic control module. The fluidic control module is configured to instruct the fluidic control system to (a) flow different sample-generation components from the storage unit to the sample area and control reaction conditions at the sample area to generate a sample; and (b) flow at least one sample-analysis component from the storage unit to the sample area. Said at least one sample-analysis component is

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configured to react with the sample to provide optically detectable signals indicative of an event-of-interest. The assay system also includes an imaging system that is configured to detect the optically detectable signals from the sample. The system controller is configured to automatically generate the sample and analyze the sample by selectively controlling the fluidic device holder, the fluidic control system, and the imaging system.

In another embodiment, a method of performing a biological or chemical assay is provided. The method includes: (a) providing a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays, the reaction components including sample-generation components and sample-analysis components; (b) flowing sample generation components according to a predetermined protocol to generate a sample at the sample area; (c) selectively controlling reaction conditions at the sample area to facilitate generating the sample; (d) flowing sample-analysis components to the sample area; and (e) detecting optical signals emitted from the sample area, the optical signals being indicative of an event-of-interest between the sample-analysis components and the sample; wherein (b)-(e) are conducted in an automated manner.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of an assay system for performing biological or chemical assays formed in accordance with one embodiment.

FIG. 2 is a side view of a workstation configured to perform biological or chemical assays in accordance with one embodiment.

FIG. 3 is a front view of the workstation of FIG. 2.

FIG. 4 is a diagram of a fluidic network formed in accordance with one embodiment.

FIG. 5 is a perspective view of a flow cell formed in accordance with one embodiment.

FIG. 6 is a cross-section of the flow cell shown in FIG. 5 taken along the line 6-6 in FIG. 5.

FIG. 7 is a plan view of the flow cell of FIG. 5.

FIG. 8 is an enlarged view of a curved segment of a flow channel.

FIG. 9 is a perspective view of a fluidic device formed in accordance with one embodiment.

FIG. 10 is another perspective view of the fluidic device of FIG. 9.

FIG. 11 is a cross-section of the fluidic device of FIG. 9 taken along the lines 11-11 in FIG. 9.

FIG. 12 is a perspective view of a fluidic device formed in accordance with another embodiment.

FIG. 13 is a perspective view of the fluidic device of FIG. 12.

FIG. 14 is a plan view of a fluidic device formed in accordance with one embodiment.

FIG. 15 is a side perspective view of the fluidic device of FIG. 14.

FIG. 16 is a partially exploded view of a device holder formed in accordance with one embodiment.

FIG. 17 is a perspective view of the assembled holder of FIG. 16.

FIG. 18 is a perspective view of a support structure that may be used in the holder of FIG. 16.

FIG. 19 is a top plan view of the holder of FIG. 16.

FIG. 20 is a perspective view of the holder of FIG. 16 having a cover assembly in an open position.

FIG. 21 is an enlarged plan view of the holder of FIG. 16.



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FIG. 22 is a perspective view of a cover assembly that may be used in the holder of FIG. 16.

FIG. 23 is a cross-section of the cover assembly taken along the line 23-23 shown in FIG. 22.

FIG. 24 is a perspective view of a flow system that may be used with the holder of FIG. 16.

FIG. 25 is a block diagram of a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 26 is a block diagram illustrating a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 27 is a block diagram illustrating a method for orienting a sample area in accordance with one embodiment.

FIG. 28 is a perspective view of a fluid storage system formed in accordance with one embodiment.

FIG. 29 is a side cross-section of the fluid storage system of FIG. 28.

FIG. 30 is a perspective view of a removal assembly that may be used with the fluid storage system of FIG. 28.

FIG. 31 is a perspective view of a reaction component tray formed in accordance with one embodiment.

FIG. 32 is a top plan view of the tray shown in FIG. 31.

FIG. 33 is a side view of the tray shown in FIG. 31.

FIG. 34 is a front view of the tray shown in FIG. 31.

FIG. 35 is a side cross-section of a component well that may be used with the tray of FIG. 31.

FIG. 36 is a bottom perspective view of the component well of FIG. 35.

FIG. 37 is a perspective view of a component well that may be used with the tray of FIG. 31.

FIG. 38 is a diagram of an optical imaging system in accordance with one embodiment.

FIG. 39 is a perspective view of a motion-control system in accordance with one embodiment.

FIG. 40 is a perspective view of components that may be used with the motion-control system of FIG. 39.

FIG. 41 is a perspective view of an optical base plate that may be used in the imaging system of FIG. 38.

FIG. 42 is a plan view of the base plate of FIG. 41.

FIG. 43 is a perspective view of an optical component formed in accordance with one embodiment that may be used in the imaging system of FIG. 38.

FIG. 44 is a cut-away perspective view of the optical component of FIG. 43.

FIG. 45 is a front view of the optical component of FIG. 43.

FIG. 46 is a side view of the optical component of FIG. 43 during a mounting operation.

FIG. 47 is a block diagram illustrating a method of assembling an optical train in accordance with one embodiment.

FIG. 48 is a perspective view of a light source module formed in accordance with one embodiment.

FIG. 49 is a side view of the light source module of FIG. 48.

FIG. 50 is a plan view of the light source module of FIG. 48.

FIG. 51 is a plan view of an image-focusing system in accordance with one embodiment.

FIG. 52 is a perspective view of a rotatable mirror assembly that may be used in the image-focusing system of FIG. 51.

FIG. 53 is a schematic diagram of a rotatable mirror in an imaging position that may be used in the image-focusing system of FIG. 51.

FIGS. 54 and 55 illustrate sample images that may be obtained by the image-focusing system of FIG. 51.

FIG. 56 is a schematic diagram of the rotatable mirror of FIG. 53 in a focusing position.

FIGS. 57 and 58 illustrate test images that may be obtained by the image-focusing system of FIG. 51.

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FIG. 59 is a block diagram illustrating a method for controlling focus of an optical imaging system.

FIG. 60 illustrates a method for performing an assay for biological or chemical analysis.

FIG. 61 illustrates a method for performing an assay for biological or chemical analysis.

## DETAILED DESCRIPTION OF THE INVENTION

Embodiments described herein include various systems, methods, assemblies, and apparatuses used to detect desired reactions in a sample for biological or chemical analysis. In some embodiments, the desired reactions provide optical signals that are detected by an optical assembly. The optical signals may be light emissions from labels or may be transmission light that has been reflected or refracted by the sample. For example, embodiments may be used to perform or facilitate performing a sequencing protocol in which ssDNA is sequenced in a flow cell. In particular embodiments, the embodiments described herein can also perform an amplification protocol to generate a sample-of-interest for sequencing.

As used herein, a “desired reaction” includes a change in at least one of a chemical, electrical, physical, and optical property or quality of a substance that is in response to a stimulus. For example, the desired reaction may be a chemical transformation, chemical change, or chemical interaction. In particular embodiments, the desired reactions are detected by an imaging system. The imaging system may include an optical assembly that directs optical signals to a sensor (e.g., CCD or CMOS). However, in other embodiments, the imaging system may detect the optical signals directly. For example, a flow cell may be mounted onto a CMOS sensor. However, the desired reactions may also be a change in electrical properties. For example, the desired reaction may be a change in ion concentration within a solution.

Exemplary reactions include, but are not limited to, chemical reactions such as reduction, oxidation, addition, elimination, rearrangement, esterification, amidation, etherification, cyclization, or substitution; binding interactions in which a first chemical binds to a second chemical; dissociation reactions in which two or more chemicals detach from each other; fluorescence; luminescence; chemiluminescence; and biological reactions, such as nucleic acid replication, nucleic acid amplification, nucleic acid hybridization, nucleic acid ligation, phosphorylation, enzymatic catalysis, receptor binding, or ligand binding. The desired reaction can also be addition or elimination of a proton, for example, detectable as a change in pH of a surrounding solution or environment.

The stimulus can be at least one of physical, optical, electrical, magnetic, and chemical. For example, the stimulus may be an excitation light that excites fluorophores in a substance. The stimulus may also be a change in a surrounding environment, such as a change in concentration of certain biomolecules (e.g., enzymes or ions) in a solution. The stimulus may also be an electrical current applied to a solution within a predefined volume. In addition, the stimulus may be provided by shaking, vibrating, or moving a reaction chamber where the substance is located to create a force (e.g., centripetal force). As used herein, the phrase “in response to a stimulus” is intended to be interpreted broadly and include more direct responses to a stimulus (e.g., when a fluorophore emits energy of a specific wavelength after absorbing incident excitation light) and more indirect responses to a stimulus in that the stimulus initiates a chain of events that eventually results in the response (e.g., incorporation of a base in pyrosequencing eventually resulting in chemiluminescence). The stimulus



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may be immediate (e.g., excitation light incident upon a fluorophore) or gradual (e.g., change in temperature of the surrounding environment).

As used herein, the phrase “activity that is indicative of a desired reaction” and variants thereof include any detectable event, property, quality, or characteristic that may be used to facilitate determining whether a desired reaction has occurred. The detected activity may be a light signal generated in fluorescence or chemiluminescence. The detected activity may also be a change in electrical properties of a solution within a predefined volume or along a predefined area. The detected activity may be a change in temperature.

Various embodiments include providing a reaction component to a sample. As used herein, a “reaction component” or “reactant” includes any substance that may be used to obtain a desired reaction. For example, reaction components include reagents, enzymes, samples, other biomolecules, and buffer solutions. The reaction components are typically delivered to a reaction site (e.g., area where sample is located) in a solution or immobilized within a reaction site. The reaction components may interact directly or indirectly with the substance of interest.

In particular embodiments, the desired reactions are detected optically through an optical assembly. The optical assembly may include an optical train of optical components that cooperate with one another to direct the optical signals to an imaging device (e.g., CCD, CMOS, or photomultiplier tubes). However, in alternative embodiments, the sample region may be positioned immediately adjacent to an activity detector that detects the desired reactions without the use of an optical train. The activity detector may be able to detect predetermined events, properties, qualities, or characteristics within a predefined volume or area. For example, an activity detector may be able to capture an image of the predefined volume or area. An activity detector may be able to detect an ion concentration within a predefined volume of a solution or along a predefined area. Exemplary activity detectors include charged-coupled devices (CCD’s) (e.g., CCD cameras); photomultiplier tubes (PMT’s); molecular characterization devices or detectors, such as those used with nanopores; microcircuit arrangements, such as those described in U.S. Pat. No. 7,595,883, which is incorporated herein by reference in the entirety; and CMOS-fabricated sensors having field effect transistors (FET’s), including chemically sensitive field effect transistors (chemFET), ion-sensitive field effect transistors (ISFET), and/or metal oxide semiconductor field effect transistors (MOSFET).

As used herein, the term “optical components” includes various elements that affect the propagation of optical signals. For example, the optical components may at least one of redirect, filter, shape, magnify, or concentrate the optical signals. The optical signals that may be affected include the optical signals that are upstream from the sample and the optical signals that are downstream from the sample. In a fluorescence-detection system, upstream components include those that direct excitation radiation toward the sample and downstream components include those that direct emission radiation away from the sample. Optical components may be, for example, reflectors, dichroics, beam splitters, collimators, lenses, filters, wedges, prisms, mirrors, detectors, and the like. Optical components also include bandpass filters, optical wedges, and optical devices similar to those described herein.

As used herein, the term “optical signals” or “light signals” includes electromagnetic energy capable of being detected. The term includes light emissions from labeled biological or chemical substances and also includes transmitted light that is

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refracted or reflected by optical substrates. Optical or light signals, including excitation radiation that is incident upon the sample and light emissions that are provided by the sample, may have one or more spectral patterns. For example, more than one type of label may be excited in an imaging session. In such cases, the different types of labels may be excited by a common excitation light source or may be excited by different excitation light sources at different times or at the same time. Each type of label may emit optical signals having a spectral pattern that is different from the spectral pattern of other labels. For example, the spectral patterns may have different emission spectra. The light emissions may be filtered to separately detect the optical signals from other emission spectra.

As used herein, when the term “different” is used with respect to light emissions (including emission spectra or other emission characteristics), the term may be interpreted broadly to include the light emissions being distinguishable or differentiable. For example, the emission spectra of the light emissions may have wavelength ranges that at least partially overlap so long as at least a portion of one emission spectrum does not completely overlap the other emission spectrum. Different emission spectra may also have the same or similar wavelength ranges, but have different intensities that are differentiable. Different optical signals can be distinguished based on different characteristics of excitation light that produces the optical signals. For example, in fluorescence resonance energy transfer (FRET) imaging, the light emissions may be the same but the cause (e.g., excitation optical signals) of the light emissions may be different. More specifically, a first excitation wavelength can be used to excite a donor fluorophore of a donor-acceptor pair such that FRET results in emission from the acceptor and excitation of the acceptor directly will also result in emission from the acceptor. As such, differentiation of the optical signals can be based on observation of an emission signal in combination with identification of the excitation wavelength used to produce the emission. Different light emissions may have other characteristics that do not overlap, such as emission anisotropy or fluorescence lifetime. Also, when the light emissions are filtered, the wavelength ranges of the emission spectra may be narrowed.

The optical components may have fixed positions in the optical assembly or may be selectively moveable. As used herein, when the term “selectively” is used in conjunction with “moving” and similar terms, the phrase means that the position of the optical component may be changed in a desired manner. At least one of the locations and the orientation of the optical component may be changed. For example, in particular embodiments, a rotatable mirror is selectively moved to facilitate focusing an optical imaging system.

Different elements and components described herein may be removably coupled. As used herein, when two or more elements or components are “removably coupled” (or “removably mounted,” and other like terms) the elements are readily separable without destroying the coupled components. For instance, elements can be readily separable when the elements may be separated from each other without undue effort, without the use of a tool (i.e. by hand), or without a significant amount of time spent in separating the components. By way of example, in some embodiments, an optical device may be removably mounted to an optical base plate. In addition, flow cells and fluidic devices may be removably mounted to a device holder.

Imaging sessions include a time period in which at least a portion of the sample is imaged. One sample may undergo or be subject to multiple imaging sessions. For example, one



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sample may be subject to two different imaging sessions in which each imaging session attempts to detect optical signals from one or more different labels. As a specific example, a first scan along at least a portion of a nucleic acid sample may detect labels associated with nucleotides A and C and a second scan along at least a portion of the sample may detect labels associated with nucleotides G and T. In sequencing embodiments, separate sessions can occur in separate cycles of a sequencing protocol. Each cycle can include one or more imaging session. In other embodiments, detecting optical signals in different imaging sessions may include scanning different samples. Different samples may be of the same type (e.g., two microarray chips) or of different types (e.g., a flow cell and a microarray chip).

During an imaging session, optical signals provided by the sample are observed. Various types of imaging may be used with embodiments described herein. For example, embodiments described herein may utilize a “step and shoot” procedure in which regions of a sample area are individually imaged. Embodiments may also be configured to perform at least one of epi-fluorescent imaging and total-internal-reflection-fluorescence (TIRF) imaging. In other embodiments, the sample imager is a scanning time-delay integration (TDI) system. Furthermore, the imaging sessions may include “line scanning” one or more samples such that a linear focal region of light is scanned across the sample(s). Some methods of line scanning are described, for example, in U.S. Pat. No. 7,329,860 and U.S. Pat. Pub. No. 2009/0272914, each of which the complete subject matter is incorporated herein by reference in their entirety. Imaging sessions may also include moving a point focal region of light in a raster pattern across the sample(s). In alternative embodiments, imaging sessions may include detecting light emissions that are generated, without illumination, and based entirely on emission properties of a label within the sample (e.g., a radioactive or chemiluminescent component in the sample). In alternative embodiments, flow cells may be mounted onto an imager (e.g., CCD or CMOS) that detects the desired reactions.

As used herein, the term “sample” or “sample-of-interest” includes various materials or substances of interest that undergo an imaging session where optical signals from the material or substance are observed. In particular embodiments, a sample may include biological or chemical substances of interests and, optionally, an optical substrate or support structure that supports the biological or chemical substances. As such, a sample may or may not include an optical substrate or support structure. As used herein, the term “biological or chemical substances” may include a variety of biological or chemical substances that are suitable for being imaged or examined with the optical systems described herein. For example, biological or chemical substances include biomolecules, such as nucleosides, nucleic acids, polynucleotides, oligonucleotides, proteins, enzymes, polypeptides, antibodies, antigens, ligands, receptors, polysaccharides, carbohydrates, polyphosphates, nanopores, organelles, lipid layers, cells, tissues, organisms, and biologically active chemical compound(s) such as analogs or mimetics of the aforementioned species. Other chemical substances include labels that can be used for identification, examples of which include fluorescent labels and others set forth in further detail below.

Different types of samples may include different optical substrates or support structures that affect incident light in different manners. In particular embodiments, samples to be detected can be attached to one or more surfaces of a substrate or support structure. For example, flow cells may include one or more flow channels. In flow cells, the flow channels may be

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separated from the surrounding environment by top and bottom layers of the flow cell. Thus, optical signals to be detected are projected from within the support structure and may transmit through multiple layers of material having different refractive indices. For example, when detecting optical signals from an inner bottom surface of a flow channel and when detecting optical signals from above the flow channel, the optical signals that are desired to be detected may propagate through a fluid having an index of refraction, through one or more layers of the flow cells having different indices of refraction, and through the ambient environment having a different index of refraction.

As used herein, a “fluidic device” is an apparatus that includes one or more flow channels that direct fluid in a predetermined manner to conduct desired reactions. The fluidic device is configured to be fluidically coupled to a fluidic network of an assay system. By way of example, a fluidic device may include flow cells or lab-on-chip devices. Flow cells generally hold a sample along a surface for imaging by an external imaging system. Lab-on-chip devices may hold the sample and perform additional functions, such as detecting the desired reaction using an integrated detector. Fluidic devices may optionally include additional components, such as housings or imagers, that are operatively coupled to the flow channels. In particular embodiments, the channels may have channel surfaces where a sample is located, and the fluidic device can include a transparent material that permits the sample to be imaged after a desired reaction occurs.

In particular embodiments, the fluidic devices have channels with microfluidic dimensions. In such channels, the surface tension and cohesive forces of the liquid flowing there-through and the adhesive forces between the liquid and the surfaces of the channel have at least a substantial effect on the flow of the liquid. For example, a cross-sectional area (taken perpendicular to a flow direction) of a microfluidic channel may be about  $10\ \mu\text{m}^2$  or less.

In alternative embodiments, optical imaging systems described herein may be used to scan samples that include microarrays. A microarray may include a population of different probe molecules that are attached to one or more substrates such that the different probe molecules can be differentiated from each other according to relative location. An array can include different probe molecules, or populations of the probe molecules, that are each located at a different addressable location on a substrate. Alternatively, a microarray can include separate optical substrates, such as beads, each bearing a different probe molecule, or population of the probe molecules, that can be identified according to the locations of the optical substrates on a surface to which the substrates are attached or according to the locations of the substrates in a liquid. Exemplary arrays in which separate substrates are located on a surface include, without limitation, a BeadChip Array available from Illumina®, Inc. (San Diego, Calif.) or others including beads in wells such as those described in U.S. Pat. Nos. 6,266,459, 6,355,431, 6,770,441, 6,859,570, and 7,622,294; and PCT Publication No. WO 00/63437, each of which is hereby incorporated by reference. Other arrays having particles on a surface include those set forth in US 2005/0227252; WO 05/033681; and WO 04/024328, each of which is hereby incorporated by reference.

Any of a variety of microarrays known in the art can be used. A typical microarray contains sites, sometimes referred to as features, each having a population of probes. The population of probes at each site is typically homogenous having a single species of probe, but in some embodiments the populations can each be heterogeneous. Sites or features of an



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array are typically discrete, being separated. The separate sites can be contiguous or they can have spaces between each other. The size of the probe sites and/or spacing between the sites can vary such that arrays can be high density, medium density or lower density. High density arrays are characterized as having sites separated by less than about 15  $\mu\text{m}$ . Medium density arrays have sites separated by about 15 to 30  $\mu\text{m}$ , while low density arrays have sites separated by greater than 30  $\mu\text{m}$ . An array useful in the invention can have sites that are separated by less than 100  $\mu\text{m}$ , 50  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , 1  $\mu\text{m}$ , or 0.5  $\mu\text{m}$ . An apparatus or method of an embodiment of the invention can be used to image an array at a resolution sufficient to distinguish sites at the above densities or density ranges.

Further examples of commercially available microarrays that can be used include, for example, an Affymetrix® Gene-Chip® microarray or other microarray synthesized in accordance with techniques sometimes referred to as VLSIPST™ (Very Large Scale Immobilized Polymer Synthesis) technologies as described, for example, in U.S. Pat. Nos. 5,324,633; 5,744,305; 5,451,683; 5,482,867; 5,491,074; 5,624,711; 5,795,716; 5,831,070; 5,856,101; 5,858,659; 5,874,219; 5,968,740; 5,974,164; 5,981,185; 5,981,956; 6,025,601; 6,033,860; 6,090,555; 6,136,269; 6,022,963; 6,083,697; 6,291,183; 6,309,831; 6,416,949; 6,428,752 and 6,482,591, each of which is hereby incorporated by reference. A spotted microarray can also be used in a method according to an embodiment of the invention. An exemplary spotted microarray is a CodeLink™ Array available from Amersham Biosciences. Another microarray that is useful is one that is manufactured using inkjet printing methods such as Sure-Print™ Technology available from Agilent Technologies.

The systems and methods set forth herein can be used to detect the presence of a particular target molecule in a sample contacted with the microarray. This can be determined, for example, based on binding of a labeled target analyte to a particular probe of the microarray or due to a target-dependent modification of a particular probe to incorporate, remove, or alter a label at the probe location. Any one of several assays can be used to identify or characterize targets using a microarray as described, for example, in U.S. Patent Application Publication Nos. 2003/0108867; 2003/0108900; 2003/0170684; 2003/0207295; or 2005/0181394, each of which is hereby incorporated by reference.

Furthermore, optical systems described herein may be constructed to include various components and assemblies as described in PCT application PCT/US07/07991, entitled "System and Devices for Sequence by Synthesis Analysis", filed Mar. 30, 2007 and/or to include various components and assemblies as described in International Publication No. WO 2009/042862, entitled "Fluorescence Excitation and Detection System and Method", filed Sep. 26, 2008, both of which the complete subject matter are incorporated herein by reference in their entirety. In particular embodiments, optical systems can include various components and assemblies as described in U.S. Pat. No. 7,329,860 and WO 2009/137435, of which the complete subject matter is incorporated herein by reference in their entirety. Optical systems can also include various components and assemblies as described in U.S. patent application Ser. No. 12/638,770, filed on Dec. 15, 2009, of which the complete subject matter is incorporated herein by reference in its entirety.

In particular embodiments, methods, and optical systems described herein may be used for sequencing nucleic acids. For example, sequencing-by-synthesis (SBS) protocols are particularly applicable. In SBS, a plurality of fluorescently labeled modified nucleotides are used to sequence a plurality

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of clusters of amplified DNA (possibly millions of clusters) present on the surface of an optical substrate (e.g., a surface that at least partially defines a channel in a flow cell). The flow cells may contain nucleic acid samples for sequencing where the flow cells are placed within the appropriate flow cell holders. The samples for sequencing can take the form of single nucleic acid molecules that are separated from each other so as to be individually resolvable, amplified populations of nucleic acid molecules in the form of clusters or other features, or beads that are attached to one or more molecules of nucleic acid. Accordingly, sequencing can be carried out on an array such as those set forth previously herein. The nucleic acids can be prepared such that they comprise an oligonucleotide primer adjacent to an unknown target sequence. To initiate the first SBS sequencing cycle, one or more differently labeled nucleotides, and DNA polymerase, etc., can be flowed into/through the flow cell by a fluid flow subsystem (not shown). Either a single type of nucleotide can be added at a time, or the nucleotides used in the sequencing procedure can be specially designed to possess a reversible termination property, thus allowing each cycle of the sequencing reaction to occur simultaneously in the presence of several types of labeled nucleotides (e.g. A, C, T, G). The nucleotides can include detectable label moieties such as fluorophores. Where the four nucleotides are mixed together, the polymerase is able to select the correct base to incorporate and each sequence is extended by a single base. Nonincorporated nucleotides can be washed away by flowing a wash solution through the flow cell. One or more lasers may excite the nucleic acids and induce fluorescence. The fluorescence emitted from the nucleic acids is based upon the fluorophores of the incorporated base, and different fluorophores may emit different wavelengths of emission light. A deblocking reagent can be added to the flow cell to remove reversible terminator groups from the DNA strands that were extended and detected. The deblocking reagent can then be washed away by flowing a wash solution through the flow cell. The flow cell is then ready for a further cycle of sequencing starting with introduction of a labeled nucleotide as set forth above. The fluidic and detection steps can be repeated several times to complete a sequencing run. Exemplary sequencing methods are described, for example, in Bentley et al., Nature 456:53-59 (2008), WO 04/018497; U.S. Pat. No. 7,057,026; WO 91/06678; WO 07/123744; U.S. Pat. No. 7,329,492; U.S. Pat. No. 7,211,414; U.S. Pat. No. 7,315,019; U.S. Pat. No. 7,405,281, and US 2008/0108082, each of which is incorporated herein by reference.

In some embodiments, nucleic acids can be attached to a surface and amplified prior to or during sequencing. For example, amplification can be carried out using bridge amplification to form nucleic acid clusters on a surface. Useful bridge amplification methods are described, for example, in U.S. Pat. No. 5,641,658; U.S. Patent Publ. No. 2002/0055100; U.S. Pat. No. 7,115,400; U.S. Patent Publ. No. 2004/0096853; U.S. Patent Publ. No. 2004/0002090; U.S. Patent Publ. No. 2007/0128624; and U.S. Patent Publ. No. 2008/0009420. Another useful method for amplifying nucleic acids on a surface is rolling circle amplification (RCA), for example, as described in Lizardi et al., Nat. Genet. 19:225-232 (1998) and US 2007/0099208 A1, each of which is incorporated herein by reference. Emulsion PCR on beads can also be used, for example as described in Dressman et al., Proc. Natl. Acad. Sci. USA 100:8817-8822 (2003), WO 05/010145, or U.S. Patent Publ. Nos. 2005/0130173 or 2005/0064460, each of which is incorporated herein by reference in its entirety.



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Other sequencing techniques that are applicable for use of the methods and systems set forth herein are pyrosequencing, nanopore sequencing, and sequencing by ligation. Exemplary pyrosequencing techniques and samples that are particularly useful are described in U.S. Pat. No. 6,210,891; U.S. Pat. No. 6,258,568; U.S. Pat. No. 6,274,320 and Ronaghi, Genome Research 11:3-11 (2001), each of which is incorporated herein by reference. Exemplary nanopore techniques and samples that are also useful are described in Deamer et al., Acc. Chem. Res. 35:817-825 (2002); Li et al., Nat. Mater. 2:611-615 (2003); Soni et al., Clin Chem. 53:1996-2001 (2007) Healy et al., Nanomed. 2:459-481 (2007) and Cockroft et al., J. am. Chem. Soc. 130:818-820; and U.S. Pat. No. 7,001,792, each of which is incorporated herein by reference. In particular, these methods utilize repeated steps of reagent delivery. An instrument or method set forth herein can be configured with reservoirs, valves, fluidic lines and other fluidic components along with control systems for those components in order to introduce reagents and detect optical signals according to a desired protocol such as those set forth in the references cited above. Any of a variety of samples can be used in these systems such as substrates having beads generated by emulsion PCR, substrates having zero-mode waveguides, substrates having integrated CMOS detectors, substrates having biological nanopores in lipid bilayers, solid-state substrates having synthetic nanopores, and others known in the art. Such samples are described in the context of various sequencing techniques in the references cited above and further in US 2005/0042648; US 2005/0079510; US 2005/0130173; and WO 05/010145, each of which is incorporated herein by reference.

Exemplary labels that can be detected in accordance with various embodiments, for example, when present on or within a support structure include, but are not limited to, a chromophore; luminophore; fluorophore; optically encoded nanoparticles; particles encoded with a diffraction-grating; electrochemiluminescent label such as Ru(bpy)<sup>32+</sup>; or moiety that can be detected based on an optical characteristic. Fluorophores that may be useful include, for example, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malachite green, Cy3, Cy5, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, alexa dyes, phycoerythrin, bodipy, and others known in the art such as those described in Haugland, Molecular Probes Handbook, (Eugene, Oreg.) 6th Edition; The Synthesgen catalog (Houston, Tex.), Lakowicz, Principles of Fluorescence Spectroscopy, 2nd Ed., Plenum Press New York (1999), or WO 98/59066, each of which is hereby incorporated by reference. In some embodiments, the one pair of labels may be excitable by a first excitation wavelength and another pair of labels may be excitable by a second excitation wavelength.

Although embodiments are exemplified with regard to detection of samples that include biological or chemical substances supported by an optical substrate, it will be understood that other samples can be imaged by the embodiments described herein. Other exemplary samples include, but are not limited to, biological specimens such as cells or tissues, electronic chips such as those used in computer processors, and the like. Examples of some of the applications include microscopy, satellite scanners, high-resolution reprographics, fluorescent image acquisition, analyzing and sequencing of nucleic acids, DNA sequencing, sequencing-by-synthesis, imaging of microarrays, imaging of holographically encoded microparticles and the like.

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FIG. 1 is a block diagram of an assay system 100 for biological or chemical analysis formed in accordance with one embodiment. In some embodiments, the assay system 100 is a workstation that may be similar to a bench-top device or desktop computer. For example, at least a majority of the systems and components for conducting the desired reactions can be within a common housing 117 of the assay system 100. In other embodiments, the assay system 100 includes one or more components, assemblies, or systems that are remotely located from the assay system 100 (e.g., a remote database). The assay system 100 may include various components, assemblies, and systems (or sub-systems) that interact with each other to perform one or more predetermined methods or assay protocols for biological or chemical analysis.

For example, the assay system 100 includes a system controller 102 that may communicate with the various components, assemblies, and systems (or sub-systems) of the assay system 100. As shown, the assay system 100 has an optical assembly 104, an excitation source assembly 106, a detector assembly 108, and a fluidic device holder 110 that supports one or more fluidic devices 112 having a sample thereon. The fluidic device may be a flow cell, such as the flow cell 200 described below, or the fluidic device 112 may be the fluidic device 300 described below.

In some embodiments, the optical assembly 104 is configured to direct incident light from the excitation source assembly 106 onto the fluidic device(s) 112. The excitation source assembly 106 may include one or more excitation light sources that are configured to excite labels associated with the sample. The excitation source assembly 106 may also be configured to provide incident light that is reflected and/or refracted by the samples. As shown, the samples may provide optical signals that include light emissions 116 and/or transmission light 118. The device holder 110 and the optical assembly 104 may be moved relative to each other. In some embodiments, the device holder 110 includes a motor assembly 132 that moves the fluidic device 112 with respect to the optical assembly 104. In other embodiments, the optical assembly 104 may be moved in addition to or alternatively to the device holder 110. The optical assembly 104 may also be configured to direct the light emissions 116 and/or transmission light 118 to the detector assembly 108. The detector assembly 108 may include one or more imaging detectors. The imaging detectors may be, by way of example only, CCD or CMOS cameras, or photomultiplier tubes.

Also shown, the assay system 100 may include a fluidic control system 134 to control the flow of fluid throughout a fluidic network 135 (indicated by the solid lines) of the assay system 100. The fluidic control system 134 may deliver reaction components (e.g., reagents) or other fluids to the fluidic device 112 during, for example, a sequencing protocol. The assay system 100 may also include a fluid storage system 136 that is configured to hold fluids that may be used by the assay system 100 and a temperature control system 138 that regulates the temperature of the fluid. The temperature control system 138 may also generally regulate a temperature of the assay system 100 using, for example, thermal modules, heat sinks, and blowers.

Also shown, the assay system 100 may include a user interface 140 that interacts with the user. For example, the user interface 140 may include a display 142 to display or request information from a user and a user input device 144 to receive user inputs. In some embodiments, the display 142 and the user input device 144 are the same device (e.g., touchscreen). As will be discussed in greater detail below, the assay system 100 may communicate with various components to perform the desired reactions. The assay system 100



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may also be configured to analyze the detection data to provide a user with desired information.

The fluidic control system **134** is configured to direct and regulate the flow of one or more fluids through the fluidic network **135**. The fluidic control system **134** may include, for example, pumps and valves that are selectively operable for controlling fluid flow. The fluidic network **135** may be in fluid communication with the fluidic device **112** and the fluid storage system **136**. For example, select fluids may be drawn from the fluid storage system **136** and directed to the fluidic device **112** in a controlled manner, or the fluids may be drawn from the fluidic device **112** and directed toward, for example, a waste reservoir in the fluid storage system **136**. Although not shown, the fluidic control system **134** may also include flow sensors that detect a flow rate or pressure of the fluids within the fluidic network. The sensors may communicate with the system controller **102**.

The temperature control system **138** is configured to regulate the temperature of fluids at different regions of the fluidic network **135**, the fluid storage system **136**, and/or the fluidic device **112**. For example, the temperature control system **138** may include a thermocycler **113** that interfaces with the fluidic device **112** and controls the temperature of the fluid that flows along the fluidic device **112**. Although not shown, the temperature control system **138** may include sensors to detect the temperature of the fluid or other components. The sensors may communicate with the system controller **102**.

The fluid storage system **136** is in fluid communication with the fluidic device **112** and may store various reaction components or reactants that are used to conduct the desired reactions therein. The fluid storage system **136** may store fluids for washing or cleaning the fluidic network **135** or the fluidic device **112** and also for diluting the reactants. For example, the fluid storage system **136** may include various reservoirs to store reagents, enzymes, other biomolecules, buffer solutions, aqueous, and non-polar solutions, and the like. Furthermore, the fluid storage system **136** may also include waste reservoirs for receiving waste products.

The device holder **110** is configured to engage one or more fluidic devices **112**, for example, in at least one of a mechanical, electrical, and fluidic manner. The device holder **110** may hold the fluidic device(s) **112** in a desired orientation to facilitate the flow of fluid through the fluidic device **112** and/or imaging of the fluidic device **112**.

The system controller **102** may include any processor-based or microprocessor-based system, including systems using microcontrollers, reduced instruction set computers (RISC), application specific integrated circuits (ASICs), field programmable gate array (FPGAs), logic circuits, and any other circuit or processor capable of executing functions described herein. The above examples are exemplary only, and are thus not necessarily intended to limit the definition and/or meaning of the term system controller. In the exemplary embodiment, the system controller **102** executes a set of instructions that are stored in one or more storage elements, memories, or modules in order to at least one of obtain and analyze detection data. Storage elements may be in the form of information sources or physical memory elements within the assay system **100**.

The set of instructions may include various commands that instruct the assay system **100** to perform specific operations such as the methods and processes of the various embodiments described herein. The set of instructions may be in the form of a software program. As used herein, the terms “software” and “firmware” are interchangeable, and include any computer program stored in memory for execution by a computer, including RAM memory, ROM memory, EPROM

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memory, EEPROM memory, and non-volatile RAM (NVRAM) memory. The above memory types are exemplary only, and are thus not limiting as to the types of memory usable for storage of a computer program.

The software may be in various forms such as system software or application software. Further, the software may be in the form of a collection of separate programs, or a program module within a larger program or a portion of a program module. The software also may include modular programming in the form of object-oriented programming. After obtaining the detection data, the detection data may be automatically processed by the assay system **100**, processed in response to user inputs, or processed in response to a request made by another processing machine (e.g., a remote request through a communication link).

The system controller **102** may be connected to the other components or sub-systems of the assay system **100** via communication links (indicated by dashed lines). The system controller **102** may also be communicatively connected to off-site systems or servers. The communication links may be hardwired or wireless. The system controller **102** may receive user inputs or commands, from the user interface **140**. The user input device **144** may include a keyboard, mouse, a touch-screen panel, and/or a voice recognition system, and the like. Alternatively or in addition, the user input device **144** may also be the display **142**.

FIG. **1** also illustrates a block diagram of the system controller **102**. In one embodiment, the system controller **102** includes one or more processors or modules that can communicate with one another. The system controller **102** is illustrated conceptually as a collection of modules, but may be implemented utilizing any combination of dedicated hardware boards, DSPs, processors, etc. Alternatively, the system controller **102** may be implemented utilizing an off-the-shelf PC with a single processor or multiple processors, with the functional operations distributed between the processors. As a further option, the modules described below may be implemented utilizing a hybrid configuration in which certain modular functions are performed utilizing dedicated hardware, while the remaining modular functions are performed utilizing an off-the-shelf PC and the like. The modules also may be implemented as software modules within a processing unit.

The system controller **102** may include a plurality of modules **151-158** that communicate with a system control module **150**. The system control module **150** may communicate with the user interface **140**. Although the modules **151-158** are shown as communicating directly with the system control module **150**, the modules **151-158** may also communicate directly with each other, the user interface **140**, or the other systems. Also, the modules **151-158** may communicate with the system control module **150** through the other modules.

The plurality of modules **151-158** include system modules **151-153** that communicate with the sub-systems. The fluidic control module **151** may communicate with the fluidic control system **134** to control the valves and flow sensors of the fluidic network **135** for controlling the flow of one or more fluids through the fluidic network **135**. The fluid storage module **152** may notify the user when fluids are low or when the waste reservoir must be replaced. The fluid storage module **152** may also communicate with the temperature control module **153** so that the fluids may be stored at a desired temperature.

The plurality of modules **151-158** may also include an image analysis module **158** that receives and analyzes the detection data (e.g., image data) from the detector assembly **108**. The processed detection data may be stored for subse-



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quent analysis or may be transmitted to the user interface **140** to display desired information to the user. Protocol modules **155-157** communicate with the system control module **150** to control the operation of the sub-systems when conducting predetermined assay protocols. The protocol modules **155-157** may include sets of instructions for instructing the assay system **100** to perform specific operations pursuant to predetermined protocols.

The protocol module **155** may be configured to issue commands for generating a sample within the fluidic device **112**. For example, the protocol module **155** may direct the fluid storage system **136** and the temperature control system **138** to generate the sample in a sample area. In one particular embodiment, the protocol module **155** may issue commands to perform bridge PCR where clusters of clonal amplicons are formed on localized areas within a channel (or lane) of a flow cell.

The protocol module **156** may be a sequencing-by-synthesis (SBS) module configured to issue various commands for performing sequencing-by-synthesis processes. In some embodiments, the SBS module **156** may also process detection data. After generating the amplicons through bridge PCR, the SBS module **156** may provide instructions to linearize or denature the amplicons to make sstDNA and to add a sequencing primer such that the sequencing primer may be hybridized to a universal sequence that flanks a region of interest. Each sequencing cycle extends the sstDNA by a single base and is accomplished by modified DNA polymerase and a mixture of four types of nucleotides delivery of which can be instructed by the SBS module **156**. The different types of nucleotides have unique fluorescent labels, and each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in each cycle. After a single base is added to the sstDNA, the SBS module **156** may instruct a wash step to remove nonincorporated nucleotides by flowing a wash solution through the flow cell. The SBS module **156** may further instruct the excitation source assembly and detector assembly to perform an image session(s) to detect the fluorescence in each of the four channels (i.e., one for each fluorescent label). After imaging, the SBS module **156** may instruct delivery of a deblocking reagent to chemically cleave the fluorescent label and the terminator from the sstDNA. The SBS module **156** may instruct a wash step to remove the deblocking reagent and products of the deblocking reaction. Another similar sequencing cycle may follow. In such a sequencing protocol, the SBS module **156** may instruct the fluidic control system **134** to direct a flow of reagent and enzyme solutions through the fluidic device **112**.

In some embodiments, the SBS module **157** may be configured to issue various commands for performing the steps of a pyrosequencing protocol. Pyrosequencing detects the release of inorganic pyrophosphate (PPi) as particular nucleotides are incorporated into the nascent strand (Ronaghi, M. et al. (1996) "Real-time DNA sequencing using detection of pyrophosphate release." *Analytical Biochemistry* 242(1), 84-9; Ronaghi, M. (2001) "Pyrosequencing sheds light on DNA sequencing." *Genome Res.* 11(1), 3-11; Ronaghi, M. et al. (1998) "A sequencing method based on real-time pyrophosphate." *Science* 281(5375), 363; U.S. Pat. No. 6,210,891; U.S. Pat. No. 6,258,568 and U.S. Pat. No. 6,274,320, the disclosures of which are incorporated herein by reference in their entireties. In pyrosequencing, released PPi can be detected by being immediately converted to adenosine triphosphate (ATP) by ATP sulfurylase, and the level of ATP generated is detected via luciferase-produced photons. In this case, the fluidic device **112** may include millions of wells where each well has a single capture bead having clonally

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amplified sstDNA thereon. Each well may also include other smaller beads that, for example, may carry immobilized enzymes (e.g., ATP sulfurylase and luciferase) or facilitate holding the capture bead in the well. The SBS module **157** may be configured to issue commands to the fluidic control module **151** to run consecutive cycles of fluids that carry a single type of nucleotide (e.g., 1st cycle: A; 2nd cycle: G; 3rd cycle: C; 4th cycle: T; 5th cycle: A; 6th cycle: G; 7th cycle: C; 8th cycle: T; and on). When a nucleotide is incorporated into the DNA, pyrophosphate is released thereby instigating a chain reaction where a burst of light is generated. The burst of light may be detected by a sample detector of the detector assembly. Detection data may be communicated to the system control module **150**, the image analysis module **158**, and/or the SBS module **157** for processing. The detection data may be stored for later analysis or may be analyzed by the system controller **102** and an image may be sent to the user interface **140**.

In some embodiments, the user may provide user inputs through the user interface **140** to select an assay protocol to be run by the assay system **100**. In other embodiments, the assay system **100** may automatically detect the type of fluidic device **112** that has been inserted into the device holder **110** and confirm with the user the assay protocol to be run. Alternatively, the assay system **100** may offer a limited number of assay protocols that could be run with the determined type of fluidic device **112**. The user may select the desired assay protocol, and the assay system **100** may then perform the selected assay protocol based on preprogrammed instructions.

FIGS. 2 and 3 illustrate a workstation **160** formed in accordance with one embodiment that is configured for biological and chemical analysis of a sample. As shown, the workstation **160** is oriented with respect to mutually perpendicular X, Y, and Z-axes. In the illustrated embodiment, a gravitational force *g* extends parallel to the Z-axis. The workstation **160** may include a workstation casing **162** (or workstation housing) that is shown in phantom in FIGS. 2 and 3. The casing **162** is configured to hold the various elements of the workstation **160**. For example, the workstation **160** may include similar elements as described above with respect to the assay system **100** (FIG. 1). As shown, the workstation **160** has an optical deck **164** having a plurality of optical components mounted thereto. The optical components may be part of an optical assembly, such as the optical assembly **602** described with reference to FIG. 38 et al. The optical deck **164** may have a fixed position with respect to the casing **162**.

The workstation **160** may also include a sample deck **166** that is movably coupled to the optical deck **164**. The sample deck **166** may have a slidable platform **168** that supports a fluidic device thereon having a sample-of-interest. In the illustrated embodiment, the fluidic device is the fluidic device **300** that is described in greater detail below. The platform **168** is configured to slide with respect to the optical deck **166** and, more specifically, with respect to an imaging lens of the optical assembly **602**. To this end, the platform **168** may slide bi-directionally along the X-axis so that the fluidic device **300** may be positioned on the sample deck **166** and so that the imaging lens may slide over the fluidic device **300** to image the sample therein. In other embodiments, the platform **168** may be stationary and the sample deck **166** may slide bi-directionally along the X-axis to position the fluidic device **300** with respect to an imaging lens of the optical assembly **602**. Thus, the platform and sample deck can be moveable relative to each other due to movement of the sample deck, platform, or both.



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Also shown, the workstation **160** may include a user interface **172**, a computing system **174** (FIG. 2), and fluid storage units **176** and **178** (FIG. 4). The user interface **172** may be a touchscreen that is configured to display information to a user and also receive user inputs. For example, the touchscreen may receive commands to perform predetermined assay protocols or receive inquiries from the user. The computing system **174** may include processors and modules, such as the system controller **102** and the modules **151-158** described with reference to FIG. 1. The fluid storage units **176** and **178** may be part of a larger fluid storage system. The fluid storage unit **176** may be for collecting waste that results from performing the assays and the fluid storage unit **178** may include a buffer solution.

FIG. 4 is a diagram of a fluidic network **552** that may be used in the workstation **160** (FIG. 2). As used herein, fluids may be liquids, gels, gases, or a mixture of thereof. Also, a fluid can be a mixture of two or more fluids. The fluidic network **552** may include a plurality of fluidic components (e.g., fluid lines, pumps, flow cells or other fluidic devices, manifolds, reservoirs) configured to have one or more fluids flowing therethrough. As shown, the fluidic network **552** includes a plurality of fluidic components **553-561** interconnected through fluid lines (indicated by the solid lines). In the illustrated embodiment, the fluidic network **552** includes a buffer solution container **553**, a reagent tray **554**, a multi-port valve **555**, a bypass valve **556**, a flow rate sensor **557**, a flow cell **558**, another flow rate sensor **559**, a pump **560**, and a waste reservoir **561**. Fluid flow directions are indicated by arrows along the fluid lines. In addition to the fluidic components **553-561**, the fluidic network may also include other fluidic components.

The reagent tray **554** may be similar to the reaction component tray (or reaction component storage unit) **1020** described in greater detail below. The tray **1020** may include various containers (e.g., vials or tubes) containing reaction components for performing assays with embodiments described herein. Operation of the multi-port valve **555** may be controlled by an assay system, such as the assay system **100**, to selectively flow different fluids, including mixtures thereof, to the flow cell **558**. The flow cell **558** may be the flow cell **200** or the fluidic device **300**, which are described in greater detail below, or other suitable fluidic devices.

FIGS. 5-60, which are described in greater detail below, illustrate various elements (e.g., components, devices, assemblies, systems, and the like) and methods that may be used with the workstation **160**. These elements may cooperate with one another in imaging a sample, analyzing the detection data, and providing information to a user of the workstation **160**. However, the following elements and methods may also be used independently, in other apparatuses, or with other apparatuses. For example, the flow cell **200** and the fluidic device **300** may be used in other assay systems. The optical assembly **602** (and elements thereof) may be used for examining other items, such as microcircuits. Furthermore, the device holder **400** may be used to hold other fluidic devices, such as lab-on-chip devices. Assay systems with these devices may or may not be include an optical assembly to detect the desired reactions.

FIGS. 5-7 illustrate a flow cell **200** formed in accordance with one embodiment. As shown in FIGS. 5-7, the flow cell **200** is oriented relative to the X, Y, and Z-axes. The flow cell **200** is configured to hold a sample-of-interest **205** in a flow channel **206**. The sample **205** is illustrated as a plurality of DNA clusters that can be imaged during a SBS protocol, but other samples may be used in alternative embodiments. Although only the single U-shaped flow channel **206** is illus-

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trated, alternative embodiments may include flow cells having multiple flow channels with differently shaped paths. The flow cell **200** may be in fluid communication with a fluidic system (not shown) that is configured to deliver reagents to the sample **205** in the flow channel **206**. In some embodiments, the sample **205** may provide detectable characteristics (e.g., through fluorescence or chemiluminescence) after desired reactions occur. For instance, the flow cell **200** may have one or more sample areas or regions (i.e., areas or regions where the sample **205** is located) from which optical signals are emitted. In some embodiments, the flow cell **200** may also be used to generate the sample **205** for performing a biological or chemical assay. For example, the flow cell **200** may be used to generate the clusters of DNA before the SBS protocol is performed.

As shown in FIGS. 5-7, the flow cell **200** can include a first layer **202** and a second layer **204** that are secured together and define the flow channel **206** therebetween. The first layer **202** has a mounting surface **208** and an outer or exterior surface **210** (FIGS. 5 and 6). The mounting and outer surfaces **208** and **210** face in opposite directions along the Z-axis and define a thickness  $T_1$  (FIGS. 5 and 6) therebetween. The thickness  $T_1$  is substantially uniform along an XY-plane, but may vary in alternative embodiments. The second layer **204** has a channel surface **212** (FIG. 6) and an outer or exterior surface **214**. The channel and outer surfaces **212** and **214** face in opposite directions along the Z-axis and define a thickness  $T_2$  (FIG. 6) therebetween.

Also shown in FIG. 5, the first layer **202** has a dimension or length  $L_1$  measured along the X-axis and another dimension or width  $W_1$  measured along the Y-axis. In some embodiments, the flow cell **200** may be characterized as a microdevice. Microdevices may be difficult to hold or move by an individual's hands. For example, the length  $L_1$  of the flow cell **200** may be about 100 mm, or about 50 mm, or less. In particular embodiments, the length  $L_1$  is about 30 mm or less. In some embodiments, the width  $W_1$  may be about 35 mm, or about 25 mm or less or, more particularly, the width  $W_1$  may be about 15 mm or less. Furthermore, a combined or total height  $H_T$  shown in FIG. 7 (e.g., a sum of thicknesses  $T_1$  and  $T_2$ ) may be about 10 mm, or about 5 mm or less. More specifically, the height  $H_T$  may be about 2 mm or about 1.5 mm or less.

The flow cell **200** includes edges **231-234** that are linear in the illustrated embodiment. Edges **231** and **233** are spaced apart by the width  $W_1$  and extend the length  $L_1$  of the flow cell **200**. Edges **232** and **234** are spaced apart by the length  $L_1$  and extend along the width  $W_1$ . Also shown, the second layer **204** may have a dimension or length  $L_2$  measured along the X-axis and another dimension or width  $W_2$  measured along the Y-axis. In the illustrated embodiment, the edges **231-234** define a perimeter of the flow cell **200** and extend along a common cell plane that extends parallel to the XY-plane. Also shown, the second layer **204** may have edges **241-244** that are similarly oriented as the edges **231-234** as shown in FIG. 5.

In the illustrated embodiment, the width  $W_1$  is substantially greater than the width  $W_2$ , and the second layer **204** is positioned on only a portion of the mounting surface **208**. As such, the mounting surface **208** includes exposed grip portions **208A** and **208B** on opposite sides of the second layer **204**. The width  $W_2$  extends between the grip portions **208A** and **208B**. The flow cell **200** may also have cell sides **256** and **258** that face in opposite directions along the Z-axis. In the illustrated embodiment, the cell side **256** includes the grip portions **208A** and **208B** and the exterior surface **214**, and the cell side **258** includes the exterior surface **210**. Also shown, the flow cell **200** may extend lengthwise between opposite first and



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second cell ends **246** and **248**. In the illustrated embodiment, the edges **232** and **242** are substantially flush with respect to each other at the first cell end **246**, and the edges **234** and **244** are substantially flush with respect to each other at the opposite second cell end **248**.

As shown in FIG. 6, the second layer **204** has at least one grooved portion **216** that extends along the channel surface **212**. In the illustrated embodiment, the channel surface **212** is etched to form the grooved portion **216**, but the grooved portion **216** may be formed by other processes, such as machining the channel surface **212**. To assemble the flow cell **200**, the channel surface **212** of the second layer **204** is mounted onto and secured to the mounting surface **208** of the first layer **202**. For example, the channel and mounting surfaces **212** and **208** may be bonded together using an adhesive (e.g., light-activated resin) that prevents leakage from the flow cell **200**. In other embodiments, the channel and mounting surfaces **212** and **208** may be secured together by other adhesives or mechanically interlocked and/or held together. Thus, the first layer **202** is configured to cover the grooved portion **216** of the second layer **204** to form the flow channel **206**. In the illustrated embodiment, the grooved portion **216** may be a single continuous groove that extends substantially the length  $L_2$  toward the first end, curves, and then extends substantially the length  $L_2$  toward the second end. Thus, the flow channel **206** may be substantially U-shaped.

In FIGS. 5-7 the sample **205** is shown as being located along only the mounting surface **208**. However, in other embodiments, the sample **205** may be located on any surface that defines the flow channel **206**. For instance, the sample **205** may also be located on the mating surface **212** of the grooved portion **216** that partially defines the flow channel **206**.

In the illustrated embodiment, the flow channel **206** may include a plurality of channel segments **250-252**. Different channel segments may have different dimensions with respect to the immediately upstream or downstream channel segment. In the illustrated embodiment, the flow channel **206** may include a channel segment **250**, which may also be referred to as the imaging segment **250**. The channel segment **250** may have a sample area that is configured to be imaged by an imaging system (not shown). The flow channel **206** may also have channel segments **251** and **252**, which may also be referred to as non-imaging segments **250** and **252**. As shown, the channel segments **250** and **252** extend parallel to each other through the flow cell **200**. The channel segments **251** and **252** of the flow channel **206** may be sized and shaped relative to the channel segment **250** to control the flow of fluid and gases that may flow therethrough.

For example, FIG. 7 also illustrates cross-sections  $C_1$ - $C_3$  of the channel segments **250-252**, respectively, that are taken perpendicular to a flow direction  $F_1$ . In some embodiments, the cross-sections  $C_1$ - $C_3$  may be differently sized (i.e., different cross-sectional areas) to control the flow of fluid through the flow channel **206**. For example, the cross-section  $C_1$  is greater in size than the cross-sections  $C_2$  and  $C_3$ . More specifically, the channel segments **250-252** of the flow channel **206** may have a substantially equal height  $H_1$  measured between the grooved portion **216** of the channel surface **212** (FIG. 6) and the mounting surface **208**. However, the channel segments **250-252** of the flow channel **206** may have different widths  $W_3$ - $W_5$ , respectively. The width  $W_3$  is greater than the widths  $W_4$  and  $W_5$ . The channel segment **251** may constitute a curved or elbow segment that fluidically joins the channel segments **250** and **252**. The cross-section  $C_3$  is smaller than the cross-sections  $C_1$  and  $C_2$ . For example, the width  $W_5$  is less than the widths  $W_3$  and  $W_4$ .

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FIG. 8 is an enlarged view of the curved segment **251** and portions of the channel segments **250** and **252**. As described above, the channel segments **250** and **252** may extend parallel to each other. Within the flow channel **206**, it may be desirable to have a uniform flow across the sample area. For example, the fluid may include stream portions  $F_2$ - $F_4$ . Dimensions of the channel segments **250-252** may be configured so that the stream portions  $F_2$ - $F_4$  have substantially equal flow rates across the sample area. In such embodiments, different sections or portions of the sample **205** (FIG. 5) may have a substantially equal amount of time to react with reaction components within the fluid.

To this end, the curved segment **251** of the flow channel **206** may have a non-continuous contour that fluidically joins the channel segments **250** and **252**. For example, as shown in FIG. 8, the curved segment **251** may include a tapering portion **270**, an intermediate portion **276**, and a downstream portion **278**. As shown, the tapering portion **270** has a width  $W_{5A}$  that gradually reduces in size. More specifically, the curved segment **251** may include sidewalls **272** and **274** that extend inward toward each other at a substantially equal angle. The intermediate portion **276** curves from the tapering portion **270** to the downstream portion **278**. The intermediate portion **276** has a width  $W_{5B}$  that reduces in size and then begins to increase in size. The downstream portion **278** has a substantially uniform width  $W_{5C}$  throughout and extends along a substantially linear path from the intermediate portion **276** to the channel segment **252**. In other words, the sidewalls **272** and **274** may extend parallel to each other throughout the downstream portion **278**.

Returning to FIG. 7, the flow cell **200** includes inlet and outlet ports **222** and **224**, respectively. The inlet and outlet ports **222** and **224** are formed only through the second layer **204**. However, in alternative embodiments, the inlet and outlet ports **222** and **224** may be formed through only the first layer **202** or through both layers **202** and **204**. The flow channel **206** is in fluid communication with and extends between the inlet and outlet ports **222** and **224**. In particular embodiments, the inlet and outlet ports **222** and **224** are located proximate to each other at the cell end **248** of the flow cell **200** (or proximate to the edges **234** and **244**). For example, a spacing **282** that separates the inlet and outlet ports **222** and **224** may be approximately equal to the width  $W_3$ . More specifically, the spacing **282** may be about 3 mm, about 2 mm, or less. Furthermore, the channel segments **250** and **252** may be separated by a spacing **280**. The spacing **280** may be less than the width  $W_3$  of the channel segment **250** or, more particularly, less than the width  $W_4$  of the channel segment **252**. Thus, a path of the flow channel **206** may be substantially U-shaped and, in the illustrated embodiment, have a non-continuous contour along the curved segment **251**.

In alternative embodiments, the flow channel **206** may have various paths such that the inlet and outlet ports **222** and **224** have different locations in the flow cell **200**. For example, the flow channel may form a single lane that extends from the inlet port at one end of the flow cell to the outlet port at the opposite end of the flow cell.

With respect to FIG. 6, in some embodiments, the thickness  $T_2$  (FIG. 6) of the second layer **204** is substantially uniform along the imaging portion **250**. The uniform thickness  $T_2$  along the imaging portion **250** may be configured to transmit optical signals therethrough. Furthermore, the thickness  $T_1$  of the first layer **202** is substantially uniform along the imaging portion **250** and configured to permit uniform transfer of thermal energy therethrough into the flow channel **206**.

FIGS. 9-11 illustrate a fluidic device **300** formed in accordance with one embodiment. For illustrative purposes, the



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fluidic device **300** is oriented with respect to the mutually perpendicular X, Y, and Z-axes shown in FIGS. **9** and **10**. FIGS. **9** and **10** are perspective views of the fluidic device **300**. As shown in FIGS. **9** and **10**, the fluidic device **300** includes a cartridge (or flow cell carrier) **302** and the flow cell **200**. The cartridge **302** is configured to hold the flow cell **200** and facilitate orienting the flow cell **200** for an imaging session.

In some embodiments, the fluidic device **300** and the cartridge **302** may be removable such that the cartridge **302** may be removed from an imaging system (not shown) by an individual or machine without damage to the fluidic device **300** or cartridge **302**. For example, the cartridge **302** may be configured to be repeatedly inserted and removed into the imaging system without damaging the cartridge **302** or rendering the cartridge **302** unsuitable for its intended purpose. In some embodiments, the fluidic device **300** and the cartridge **302** may be sized and shaped to be handheld by an individual. Furthermore, the fluidic device **300** and the cartridge **302** may be sized and shaped to be carried by an automated system.

As shown in FIGS. **9** and **10**, the cartridge **302** may include a housing or carrier frame **304** and a cover member **306** that is coupled to the housing **304**. The housing **304** has housing or carrier sides **303** and **305** that face in opposite directions along the Z-axis and have a height  $H_2$  (shown in FIG. **11**) extending therebetween. As shown in FIG. **9**, the housing **304** includes a bridge member **324** at a loading end **316** of the fluidic device **300** and a base member **326** at an opposite receiving end **318** of the fluidic device **300**. The housing **304** also includes a pair of spaced apart leg extensions **328** and **330** that extend between the bridge and base members **324** and **326**. The bridge member **324** extends between and joins the leg extensions **328** and **330**. The bridge member **324** may include a recess **321** (shown in FIG. **10**) that opens to an exterior of the fluidic device **300**. As shown in FIG. **9**, the leg extensions **328** and **330** may have a plurality of grip members **371-374** that are configured to grip the cell side **256** of the flow cell **200**.

Also shown in FIG. **9**, the fluidic device **300** may have a device window **315** that passes entirely through the cartridge **302** along the Z-axis. In the illustrated embodiment, the device window **315** is substantially framed by the bridge member **324**, the cover member **306**, and the leg extensions **328** and **330**. The device window **315** includes a reception space **308** and a plurality of recesses **320** and **322** that are immediately adjacent to the reception space **308**. The reception space **308** is configured to receive the flow cell **200**. When the flow cell **200** is positioned within the reception space **308**, the flow cell **200** is exposed to an exterior of the fluidic device **300** such that the flow cell **200** may be viewed or directly engaged along the housing side **303** and also the housing side **305**. For example, the cell side **258** (also shown in FIG. **11**) that faces in an opposite direction along the Z-axis relative to the cell side **256**. The cell side **256** may be viewed by the imaging system or directly engaged by another component along the housing side **303**. Likewise, the cell side **258** may be viewed by the imaging system or directly engaged by another component along the housing side **305**.

With respect to FIGS. **9** and **10**, the cover member **306** may include a cover body **340** and a gasket **342** that are coupled to each other. The gasket **342** includes inlet and outlet passages **346** and **344** (shown in FIG. **9**) that are located proximate to one another. In the illustrated embodiment, the cover body **340** and the gasket **342** are co-molded into a unitary structure. When formed, the cover body **340** and the gasket **342** may have different compressible properties. For example, in particular embodiments, the gasket **342** may comprise a material that is more compressible than material of the cover body **340**.

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However, in alternative embodiments, the cover body **340** and the gasket **342** may be separate parts that are coupled together (e.g., mechanically or using an adhesive). In other embodiments, the cover body **340** and the gasket **342** may be different portions or regions of a single continuous structure.

The cover member **306** may be movably coupled to the housing **304**. For example, the cover member **306** may be rotatably coupled to the base member **326** of the housing **304**. In such embodiments, the gasket **342** is rotatable about an axis of rotation  $R_1$  between a mounted position (shown in FIG. **9**) and a disengaged position (shown in FIG. **10**). In other embodiments in which the cover member **306** is movably coupled to the housing **304**, the cover member **306** may be detachable from the housing **304**. For example, when attached to the housing **304**, the detachable cover member may be in a mounted position that is similar to the mounted position as shown in FIG. **9**. When unattached to the housing **304**, the detachable cover member may be completely removed in a disengaged position.

Also shown in FIG. **10**, the housing **304** may define a cartridge cavity **338** (FIG. **10**) that is accessible when the cover member **306** is in the disengaged position. In some embodiments, an identification transmitter **336** may be positioned within the cartridge cavity **338**. The identification transmitter **336** is configured to communicate information about the flow cell **200** to a reader. For example, the identification transmitter **336** may be an RFID tag. The information provided by the identification transmitter **336** may, for example, identify the sample in the flow cell **200**, a lot number of the flow cell or sample, a date of manufacture, and/or the assay protocol to be performed when the flow cell **200** is inserted into the imaging system. The identification transmitter **336** may communicate other information as well.

FIG. **11** is a cross-section of the fluidic device **300** viewed along the Y-axis. In some embodiments, the reception space **308** is sized and shaped relative to the flow cell **200** so that the flow cell **200** is retained in the space, but in at least some configurations may float therein. As used herein, the term “float” and like terms includes the component being permitted to move a limited distance in at least one direction (e.g., along the X, Y, or Z-axes). For example, the flow cell **200** may be capable of shifting within the reception space **308** along the XY-plane. The flow cell **200** may also be capable of moving in a direction along the Z-axis within the reception space **308**. Furthermore, the flow cell **200** can also be capable of slightly rotating within the reception space **308**. In particular embodiments, the housing **304** permits the flow cell **200** to shift, move, and slightly rotate within the reception space **308** with respect to any of the X, Y, and Z-axes.

In some embodiments, the reception space **308** may also be characterized as the space that the fluidic device **300** allows the flow cell **200** to move freely within when the fluidic device **300** is holding the flow cell **200**. Thus, dimensions of the reception space **308** may be based upon positions of reference surfaces of the fluidic device **300** that can directly engage the flow cell **200**. The reference surfaces may be surfaces of the housing **304** or the cover member **306**, including the gasket **342**. For example, FIG. **11** illustrates a plurality of reference surfaces **381-387**. The reference surfaces **381** and **382** of the grip members **371** and **372**, respectively, and the reference surface **383** of the gasket **342** may limit movement of the flow cell **200** beyond a predetermined level when the flow cell **200** is held within the reception space **308**. The reference surface **384** of the gasket **342** and the reference surface **385** of the bridge member **324** may limit movement of the flow cell **200** along the XY-plane. Furthermore, the reference surfaces **386** and **387** of the bridge member **324** and the cover member **306**,



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respectively, may also limit movement of the flow cell **200** along the Z-axis. However, the reference surfaces **381-387** are exemplary only and the fluidic device **300** may have other reference surfaces that limit movement of the flow cell **200**.

To assemble the fluidic device **300**, the flow cell **200** may be loaded into the reception space **308**. For example, the flow cell **200** may be advanced toward the device window **315** along the housing side **305**. The edge **234** (FIG. **5**) may be advanced between the grip members **372** and **373** and the gasket **342**. The cell side **256** may then be rotated toward the grip members **371-374** so that the grip members **371-374** interface the cell side **256**. The edge **232** (FIG. **5**) may then be moved toward the bridge member **324** and, more specifically, the reference surface **385** of the bridge member **324**. In some embodiments, the bridge member **324** may be deflected or bent to provide more space for positioning the cell end **246** (Figures) thereon. When the flow cell **200** is loaded into the cartridge **302**, the housing **304** and the cover member **306** may effectively grip the perimeter of the flow cell **200** such that the flow cell **200** is confined to move only within the reception space **308**.

In alternative embodiments, the cell end **246** may be first inserted positioned by the bridge member **324** and then the gasket **342**. In other embodiments, the flow cell **200** may approach the housing side **303**. The grip members **371-374** may have tapered or beveled surfaces that permit the flow cell **200** to be snapped into position within the reception space **308**.

Before, after, or during the loading of the flow cell **200**, the cover member **306** may be moved to the disengaged position so that the identification transmitter **336** (FIG. **10**) may be positioned with the cartridge cavity **338** (FIG. **10**). When the gasket **342** is in the mounted position, the inlet and outlet passages **346** and **344** may have a predetermined location and orientation with respect to the housing **304** and the reception space **308**. The gasket **342** may be mounted over the flow cell **200** along an exposed portion of the flow cell **200** (i.e., the cell side **256**). The inlet and outlet passages **346** and **344** may be generally aligned with the inlet and outlet ports **224** and **222** (FIG. **5**).

However, it should be noted that the illustrated fluidic device **300** is only one particular embodiment, and the fluidic device **300** may have different configurations in alternative embodiments. For example, in alternative embodiments, the flow cell **200** may not be exposed to the exterior of the fluidic device **300** along each of the housing sides **303** and **305**. Instead, the flow cell **200** may be exposed to the exterior along only one of the housing sides (e.g., the housing side **303**). Furthermore, in alternative embodiments, the cover member **306** may not be rotatably coupled to the housing **304**. For example, the cover member **306** may be entirely detachable.

FIGS. **12-15** illustrate fluidic devices **900** and **920** formed in accordance with alternative embodiments that may also be used in assay systems, such as the assay system **100** (FIG. **1**) and the workstation **160** (FIG. **2**). The fluidic devices **900** and **920** may include similar features as the fluidic device **300**. For example, as shown, in FIGS. **12** and **13**, the fluidic device **900** may include a cartridge (or flow cell carrier) **902** and the flow cell **200**. The cartridge **902** is configured to hold the flow cell **200** and facilitate orienting the flow cell **200** for an imaging session. The cartridge **902** includes a housing **904** and a cover member **906** that is movably mounted to the housing **904**. The cover member **906** is in the mounted position in FIG. **12** and the disengaged position in FIG. **13**.

Also shown in FIGS. **12** and **13**, the fluidic device **900** may include a sealing member **910** that covers the inlet and outlet ports **222** and **224** (FIG. **13**) of the flow cell **200**. In some

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embodiments, the sealing member **910** is configured to facilitate retaining fluid within the flow channel **206** so that the sample **205** (FIG. **5**) within the flow channel **206** remains in a fluid environment. However, in some embodiments, the sealing member **910** may be configured to prevent unwanted materials from entering the flow channel **206**. As shown in FIGS. **12** and **13**, the sealing member **910** is a single piece of tape that extends between the cell ends **246** and **248** (FIG. **13**). An overhang portion **912** may extend away from the cell end **246**. In alternative embodiments, the sealing member **910** may be more than one piece of tape (e.g., one piece of tape for each of the inlet and outlet ports **222** and **224**) or the sealing member **910** may be other elements capable of covering the inlet and outlet ports **222** and **224**. For example, the sealing member **910** could include plugs.

In some embodiments, the sealing member **910** covers the inlet and outlet ports **222** and **224** when the fluidic device **900** is not mounted to an assay system. For example, the sealing member **910** may be used when the fluidic device **900** is being stored or transported, or when a sample is being grown or generated within the flow cell **200**. In such instances, the sealing member **910** may be secured to the flow cell **200** and the housing **904** as shown in FIG. **13**. More specifically, the sealing member **910** may couple to and extend along the cell side **256** and cover the inlet and outlet ports **222** and **224**. The sealing member **910** may also couple to a base member **914** of the housing **904**. The cover member **906** may then be moved to the mounted position as shown in FIG. **12** such that the sealing member **910** is sandwiched between the inlet and outlet ports **222** and **224** and the cover member **906**. The cover member **906** may facilitate preventing the sealing member **910** from being inadvertently removed. In alternative embodiments, the sealing member **910** may cover inlet and outlet passages **916** and **918** of the cover member **906**.

FIGS. **14** and **15** illustrate the fluidic device **920**, which may also have similar features as the fluidic devices **300** and **900**. As shown, the fluidic device **920** includes a cartridge (or flow cell carrier) **922** and the flow cell **200**. The cartridge **922** includes a housing **924** and a cover member **925** that is movably mounted to the housing **924**. The cover member **925** is only shown in the mounted position in FIGS. **14** and **15**. The housing **924** and the cover member **925** may be similar to the housings **204** and **904** and the cover member **306** and **906** described above.

However, the housing **924** may also include fin projections **926** and **928**. The fin projections **926** and **928** are sized and shaped to be gripped by an individual or robotic device, such as when the fluidic device **920** is being inserted in or removed from a device holder (not shown). In some embodiments, the fin projections **926** and **928** may prevent the cover assembly (not shown) from moving to the closed position if the fluidic device **920** is not properly positioned. The fin projections **926** and **928** may include tactile features **927** and **929** that are configured to be gripped by the individual. In the illustrated embodiment, the fin projections **926** and **928** are located at a receiving end **930** of the fluidic device **920**. The cover member **925** may extend between the fin projections **926** and **928**. However, the fin projections **926** and **928** may have other locations along the cartridge **902**.

FIGS. **16-24** show various features of a fluidic device holder **400** formed in accordance with one embodiment. FIG. **16** is a partially exploded view of the holder **400**. When assembled, the holder **400** may be used to hold the fluidic device **300** (FIG. **9**) and the flow cell **200** (FIG. **5**) in a desired orientation during an imaging session. Furthermore, the holder **400** may provide an interface between the fluidic device **300** and the imaging system (not shown) in which the



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holder **400** may be configured to direct fluids through the flow cell **200** and provide or remove thermal energy from the flow cell **200**. Although the holder **400** is shown as holding the fluidic device **300**, the holder **400** may be configured to hold other fluidic devices, such as lab-on-chip devices or flow cells without cartridges.

As shown in FIG. **16**, the holder **400** may include a removable cover assembly **404** and a support structure **402**. In some embodiments, the holder **400** may also include a plate structure **406** and a movable platform **408**. The plate structure **406** is operatively coupled to the cover assembly **404** and includes an opening **410** therethrough. Likewise, the platform **408** includes an opening **412** therethrough. The support structure **402** may include a heat sink **414** and a thermal module (or thermocycler) **416** that is mounted onto the heat sink **414**. The thermal module **416** includes a base portion **418** and a pedestal **420**. When the holder **400** is assembled, the support structure **402**, the platform **408**, and the plate structure **406** are stacked with respect to each other. As such, the opening **412** is sized and shaped to receive the base portion **418**, and the opening **410** is sized and shaped to receive the pedestal **420**. When assembled, the cover assembly **404** may be operatively coupled to the plate structure **406** and the support structure **402**.

FIG. **17** shows the assembled holder **400**. In the illustrated embodiment, a panel **424** is positioned over the plate structure **406** (FIG. **16**). As shown in FIGS. **16** and **17**, the cover assembly **404** includes a cover housing **435** that is coupled to the plate structure **406**. The cover housing **435** may be substantially U-shaped having a pair of spaced apart housing legs **436** and **438** that extend in a common direction. The housing legs **436** and **438** may be rotatably coupled to the plate structure **406** at joints **437** and **439**. The cover housing **435** may also include a bridge portion **440** that extends between and joins the housing legs **436** and **438**. In this manner, the cover assembly **404** may be configured to provide a viewing space **442** (FIG. **17**). The viewing space **442** may be sized and shaped to permit an imaging lens (not shown) to move in a direction  $D_x$  (FIG. **17**) along and over the flow cell **200**.

In the illustrated embodiment, the cover assembly **404** is movable relative to the plate structure **406** or support structure **402** between an open position (shown in FIG. **16**) and a closed position (shown in FIG. **17**). In the open position, the cover assembly **404** is withdrawn or retracted to permit access to a loading region **422** (shown in FIG. **18**) of the holder **400** so that the fluidic device **300** may be removed from or inserted into the loading region **422**. In the closed position, the cover assembly **404** is mounted over the fluidic device **300**. In particular embodiments, the cover assembly **404** establishes a fluid connection with the fluidic device **300** in the closed position and presses the flow cell **200** against the support structure **402**.

As shown in FIG. **16**, in some embodiments, the holder **400** includes a coupling mechanism **450** to facilitate holding the cover assembly **404** in the closed position. For example, the coupling mechanism **450** may include an operator-controlled element **452** that includes a button **453** that is coupled to a pair of latch openings **456** and **458**. The coupling mechanism **450** also includes a pair of latch ends **454** and **455** that project away from a mating face **460** of the cover housing **435**. The cover housing **435** may be biased into the open position by spring elements **464** and **466**. When the cover assembly **404** is moved into the closed position by an individual or machine, the latch ends **454** and **455** are inserted into the latch openings **456** and **458**, respectively, and grip the operator-controlled element **452**. To move the cover assembly **404** into the open position, the individual or machine may actuate the button

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**453** by, for example, pushing the button **453** inward. Since the cover housing **435** is biased by the spring elements **464** and **466**, the cover housing **435** is rotated away from the panel **424** (FIG. **17**) about the joints **437** and **439**.

In alternative embodiments, the coupling mechanism **450** may include other elements to facilitate holding the cover assembly **404** in the closed position. For example, the latch ends **454** and **455** may be replaced by magnetic elements or elements that form an interference fit with openings.

FIG. **18** is an isolated perspective view of thermal module **416** and the heat sink **414** of the support structure **402**. The thermal module **416** may be configured to control a temperature of the flow cell **200** for predetermined periods of time. For example, the thermal module **416** may be configured to raise the temperature of the flow cell **200** so that DNA in the sample may denature. Furthermore, the thermal module **416** may be configured to remove thermal energy thereby lowering the temperature of the flow cell **200**. As shown, the pedestal **420** includes a base surface **430** that is sized and shaped to interface with the flow cell **200** (FIG. **5**). The base surface **430** faces in a direction along the Z-axis. The pedestal **420** may also include a plurality of alignment members **431-433** that are positioned around the base surface **430**. In the illustrated embodiment, the alignment members **431-433** have fixed positions with respect to the base surface **430**. The alignment members **431-433** have corresponding reference surfaces that are configured to engage the flow cell **200** and facilitate positioning the flow cell **200** for imaging. For example, the reference surfaces of the alignment members **431-433** may face in respective directions along the XY-plane and, as such, may be configured to limit movement of the flow cell **200** along the XY-plane. The support structure **402** may include at least a portion of the loading region **422**. The loading region **422** may be partially defined by the base surface **430** and the reference surfaces of the alignment members **431-433**.

FIGS. **19** and **20** illustrate an alignment assembly **470** that may be used with the holder **400** in accordance with one embodiment. FIG. **19** is a plan view of the holder **400** in which the cover housing **435** is shown in phantom to illustrate the alignment assembly **470**. FIG. **20** is a perspective view of the holder **400** in which the cover assembly **404** is in the open position. (In both FIGS. **19** and **20**, the panel **424** (FIG. **17**) has been removed for illustrative purposes.)

The fluidic device **300** is loaded into the loading region **422** in FIGS. **19** and **20**. When the fluidic device **300** is loaded, the flow cell **200** is placed onto the base surface **430** (FIG. **18**) and the alignment members **432**, **433**, and **431** are advanced through the recesses **320**, **322**, and **321** (FIGS. **9** and **10**) of the cartridge **302**. More specifically, the device window **315** (FIG. **9**) along the housing side **305** may be sized and shaped to be greater than a perimeter of the base surface **430**. As such, the cartridge **302** or housing **304** may be allowed to fall around the base surface **430**, but the flow cell **200** is prevented from falling by the base surface **430**. In this manner, the cell side **258** of the flow cell **200** may be pressed against the base surface **430** so that the thermal module **416** may control a temperature of the flow cell **200**. When the flow cell **200** is mounted on the base surface **430**, the reference surfaces **381-383** (FIG. **11**) of the cartridge **302** are pressed against the cell side **256** (FIG. **11**). At this time, a cell plane of the flow cell **200** that extends along the sample **205** may be substantially aligned with an object plane of the imaging system.

In the illustrated embodiment, when the fluidic device **300** is loaded into the loading region **422**, an identification reader of the assay system may detect information from the identification transmitter **336** (FIG. **10**). For example, the holder



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400 may include an identification reader (not shown) in the plate structure 406 proximate to the identification transmitter 336. The identification reading may occur before the cover assembly 404 is mounted onto the fluidic device 300.

With reference to FIGS. 19 and 20, the alignment assembly 470 includes various elements that cooperate together in orienting and positioning the flow cell 200 for imaging. For example, the alignment assembly 470 includes a movable locator arm 472 and an actuator 474 that is operatively coupled to the locator arm 472. As shown, the actuator 474 includes a lever 476 and a pin element 478 that is coupled to the cover housing 435. In the illustrated embodiment, the lever 476 is rotatable about an axis of rotation  $R_2$  (FIG. 19). The lever 476 may be L-shaped having a first extension 480 configured to engage the pin element 478 and a second extension 482 configured to engage the locator arm 472. The locator arm 472 is also rotatable about an axis of rotation  $R_3$  (FIG. 19) and includes a finger 484 having an engagement end 486. The alignment assembly 470 also includes a biasing element 490 (e.g., a coil spring) that engages the finger 484. The engagement end 486 is configured to engage the cartridge 302 of the fluidic device 300. In alternative embodiments, the engagement end 486 may be configured to directly engage the flow cell 200.

The alignment assembly 470 is in an engaged arrangement in FIG. 19 and in a withdrawn arrangement in FIG. 20. The locator arm 472 is in a retracted position when the alignment assembly 470 is in the withdrawn arrangement and in a biased position when the alignment assembly 470 is in the engaged arrangement. To align the flow cell 200 in the loading region 422, the alignment assembly 470 is changed from the withdrawn arrangement to the engaged arrangement. For example, when the cover housing 435 is moved to the open position shown in FIG. 20, the pin element 478 engages the first extension 480 of the lever 476 causing the lever 476 to rotate about the axis  $R_2$  in a counter-clockwise direction (as shown in FIG. 19). The cover housing 435 may be maintained in the open position by the spring elements 464 and 466 (FIG. 16). When the lever 476 is rotated, the second extension 482 rotates about the axis  $R_2$  and engages the locator arm 472. The locator arm 472 is rotated about the axis  $R_3$  in a clockwise direction (as shown in FIG. 19). When the locator arm 472 is rotated, the locator arm 472 is moved to the retracted position. When moved to the retracted position, the engagement end 486 is moved away from the reference surfaces of the alignment members 431-433.

To change the alignment assembly 470 from the withdrawn arrangement to the engaged arrangement, the cover housing 435 may be rotated toward the fluidic device 300 and mounted over the flow cell 200. When the cover housing 435 is moved toward the fluidic device 300, the pin element 478 is rotated away from the first extension 480 of the lever 476. When the second extension 482 moves away from the locator arm 472, potential energy stored in the biasing element 490 may cause the locator arm 472 to rotate in a counter-clockwise direction such that the engagement end 486 presses against the cartridge 302. As such, the locator arm 472 is moved to the biased position. When moved to the biased position, the engagement end 486 is moved toward the reference surfaces of the alignment members 431-433.

FIG. 21 is an enlarged plan view of the fluidic device 300 in the loading region 422 when the engagement end 486 of the locator arm 472 is pressed against the cartridge 302. The engagement end 486 may be configured to move within the XY-plane between the retracted and biased positions. When the engagement end 486 is moved toward the biased position and presses against the cartridge 302, the engagement end

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486 provides a force  $F_{XY}$  against the cartridge 302. The cartridge 302 may shift along the XY-plane and/or press the flow cell 200 against the reference surfaces of the alignment members 431-433. The force  $F_{XY}$  has an X-component and a Y-component. The X-component may press the flow cell 200 against the alignment member 431, and the Y-component may press the flow cell 200 against the alignment members 432 and 433. As such, the alignment member 431 may stop movement of the flow cell 200 in a direction along the X-axis, and the alignment members 432 and 433 may stop movement of the flow cell 200 in a direction along the Y-axis.

Before the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 of the cover member 306 may be approximately aligned with the inlet and outlet ports 224 and 222 (FIG. 7), respectively, of the flow cell 200. After the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 are effectively (or operatively) aligned with the inlet and outlet ports 224 and 222 so that fluid may effectively flow therethrough.

Accordingly, the cover assembly 404 may be operatively coupled to the alignment assembly 470 such that one step or action causes the alignment assembly 470 to engage the fluidic device 300. More specifically, as the cover assembly 404 is mounted over the device in the closed position, the actuator 474 moves the locator arm 472 to the biased position. In the biased position, the locator arm 472 holds the flow cell 200 against the reference surfaces of the alignment members 431-433 in a fixed position along the XY-plane. When the cover assembly 404 is in the closed position, the viewing space 442 (FIG. 17) may be located over the flow cell 200 so that an imaging lens may move along the flow cell 200 to image the flow channel 206. As the cover assembly 404 is moved to the open position, the actuator 474 moves the locator arm 472 to the retracted position. However, in the illustrated embodiment, the flow cell 200 remains in position when the locator arm 472 is retracted. Accordingly, the flow cell 200 may be floatable relative to various elements. For example, the flow cell 200 may be floatable with respect to the cover member 306 and the gasket 342 when the cover member 306 is in the mounted position. The flow cell 200 may also be floatable relative to the cover assembly 404 and the base surface 430.

In some embodiments, the alignment assembly 470 and the cover assembly 404 may operate at a predetermined sequence. For example, in particular embodiments, the locator arm 472 is configured to hold the flow cell 200 against the alignment members 431-433 in the fixed position before the cover assembly 404 reaches the closed position. When the cover assembly 404 reaches the closed position, the cover assembly 404 may facilitate pressing the flow cell 200 against the base surface 430 and also pressing the inlet and outlet passages 346 and 344 against the inlet and outlet ports 224 and 222. Generally, the alignment assembly 470 can be configured to position the flow cell 200 in the x and y dimensions after the base surface 430 positions the flow cell 200 in the z dimension. Alternatively, an alignment assembly can be configured to position the flow cell 200 first in the x and y dimensions and then in the z dimension. Thus, alignment in the x, y and z dimensions can occur sequentially and in various orders in response to a single step or motion carried out by a user.

In alternative embodiments, the alignment assembly 470 may not be operatively coupled to the cover assembly 404 as described above. Instead, the alignment assembly 470 and the cover assembly 404 may operate independently from each other. As such, an individual may be required to perform a plurality of steps to align the flow cell 200 and fluidically



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couple the flow cell 200. For example, the alignment assembly 470 can be separately actuated by an individual thereby moving the locator arm 472 to align the flow cell 200. After the flow cell 200 is aligned, the individual may then lower the cover assembly 404 onto the flow cell 200. Furthermore, the alignment assembly 470 may comprise additional and/or other components than those described above.

FIG. 22 is an isolated perspective view of the cover assembly 404 in the closed position. FIG. 22 illustrates dimensions of the viewing space 442. As shown, the cover housing 435 may have a top surface 492. The viewing space 442 may have a depth  $D_p$  that is measured from the top surface 492 to the fluidic device 300 or the flow cell 200. The viewing space 442 may also have a width  $W_6$  measured along the Y-axis and a length  $L_6$  measured along the X-axis. The dimensions of the viewing space 442 may be sized so that an imaging lens (not shown) may move therethrough over the flow cell 200. More specifically, an imaging lens may enter the viewing space 442 through an access opening 443 and move in a direction along the X-axis over the flow cell 200.

FIG. 23 is a cross-section of the cover assembly 404 taken along the line 23-23 in FIG. 22. In the illustrated embodiment, the cover assembly 404 may include a plurality of compression arms 494 and 496. The compression arms 494 and 496 are configured to provide respective compressive forces  $F_{C1}$  and  $F_{C2}$  against the housing side 303 of the fluidic device 300. In the illustrated embodiment, the compression arms 494 and 496 press against the cartridge 302. However, in alternative embodiments, the compression arms 494 and 496 may press against the flow cell 200.

The compressive forces  $F_{C1}$  and  $F_{C2}$  press the housing 304 of the fluidic device 300 thereby pressing the cell side 256 (FIG. 9) of the flow cell 200 against the thermal module 416. As such, the flow cell 200 may maintain intimate contact with the base surface 430 for transferring thermal energy therebetween. In the illustrated embodiment, the compression arms 494 and 496 operate independently of each other. For example, each of the compression arms 494 and 496 is operatively coupled to respective compression springs 495 and 497.

As shown in FIG. 23, the compression arms 494 and 496 extend toward the viewing space 442 and the loading region 422. The compression arms 494 and 496 may engage the housing side 303 when the cover assembly 404 is moved to the closed position. As the compression arms 494 and 496 press against the housing side 303, resistance from the housing side 303 may cause the compression arms 494 and 496 to rotate about axes  $R_4$  and  $R_5$ . Each of the compression springs 495 and 497 may resist the rotation of the respective compression arm thereby providing the corresponding compressive force  $F_C$  against the housing side 303. Accordingly, the compression arms 494 and 496 are independently biased relative to each other.

FIG. 24 is an isolated perspective view of a flow assembly 500 of the cover assembly 404 (FIG. 16). The flow assembly 500 includes a manifold body 502 and upstream and downstream flow lines 504 and 506. As shown in FIG. 16, the manifold body 502 may extend between the housing legs 436 and 438. Returning to FIG. 24, the flow lines 504 and 506 are mechanically and fluidically coupled to the manifold body 502 at body ports 508 and 510, respectively. The flow lines 504 and 506 also include line ends 514 and 516 that are configured to be inserted into the inlet and outlet passages 346 and 344 of the gasket 342.

As shown in FIG. 24, the flow assembly 500 is in a mounted position with respect to the gasket 342. In the mounted position, the line ends 514 and 516 are inserted into the inlet and

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outlet passages 346 and 344, respectively, so that fluid may flow through the flow cell 200. Furthermore, in the mounted position, the flow assembly 500 may press the gasket 342 (FIG. 9) against the flow cell 200 so that the fluid connection is effectively sealed. To this end, the flow assembly 500 may include biasing springs 520 and 522. The biasing springs 520 and 522 are configured to press against an interior of the cover housing 435 (FIG. 16) and provide a force  $F_{C3}$  against the gasket 342. The coupling mechanism 450 (FIG. 16) may facilitate maintaining the seal against the gasket 342.

Accordingly, the cover assembly 404 may press against the housing 304 of the fluidic device 300 at three separate compression points. More specifically, the gasket 342 may constitute a first compression point  $P_1$  (shown in FIG. 24) when engaged by the line ends 514 and 516, and the compression arms 494 and 496 may contact the fluidic device 300 at second and third compression points  $P_2$  and  $P_3$  (shown in FIG. 23). As shown in FIGS. 22-24, the three compression points  $P_1$ - $P_3$  are distributed about the flow cell 200. Moreover, the cover assembly 404 independently provides the compressive forces  $F_{C1}$ - $F_{C3}$  at the compression points  $P_1$ - $P_3$ . As such, the cover assembly 404 may be configured to provide a substantially uniform compressive force against the fluidic device 300 so that the flow cell 200 is uniformly pressed against the base surface 430 and the fluidic connection is sealed from leakage.

FIG. 25 is a block diagram of a method 530 of positioning a fluidic device for sample analysis. The method 530 includes positioning at 532 a removable fluidic device on a base surface. The fluidic device may be similar to the fluidic device 300 described above. For example, the fluidic device may include a reception space, a flow cell located within the reception space, and a gasket. The flow cell may extend along an object plane in the reception space and be floatable relative to the gasket within the object plane. The method 530 also includes moving the flow cell at 534 within the reception space while on the base surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket. The moving operation 534 may include actuating a locator arm to press the flow cell against alignment members.

FIG. 26 is a block diagram illustrating a method 540 of positioning a fluidic device for sample analysis. The fluidic device 300 may be similar to the fluidic device 300 described above. The method 540 includes providing a fluidic device at 542 having a device housing that includes a reception space and a floatable flow cell located within the reception space. The device housing may include recesses that are located immediately adjacent to the reception space. The method also includes positioning at 544 the fluidic device on a support structure having alignment members. The alignment members may be inserted through corresponding recesses. Furthermore, the method 540 may include moving the flow cell at 546 within the reception space. When the flow cell is moved within the reception space, the alignment members may engage edges of the flow cell. The moving operation 546 may include actuating a locator arm to press the flow cell against the alignment members.

FIG. 27 is a block diagram illustrating a method 550 for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes. The method 550 includes providing an alignment assembly at 552. The alignment assembly may be similar to the alignment assembly 470 described above. More specifically, the alignment assembly may include a movable locator arm that has an engagement end. The locator arm may be movable between retracted and biased positions. The method 550 also includes positioning a fluidic device at 554



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on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. Furthermore, the method **550** may include moving at **556** the locator arm to the biased position. The locator arm can press the device against the reference surfaces such that the device is held in a fixed position.

FIGS. **28-37** illustrate various features of a fluid storage system **1000** (FIG. **28**). The storage system **1000** is configured to store and regulate a temperature of various fluids that may be used during predetermined assays. The storage system **1000** may be used by the workstation **160** (FIG. **2**) and enclosed by the casing **162** (FIG. **3**). As shown in FIG. **28**, the storage system **1000** includes an enclosure **1002** having a base shell (or first shell) **1004** and a top shell (or second shell) **1006** that are coupled together and define a system cavity **1008** therebetween. The enclosure **1002** may also include a system door **1010** that is configured to open and provide access to the system cavity **1008**. Also shown, the storage system **1000** may include a temperature-control assembly **1012** that is coupled to a rear of the enclosure **1002** and a elevator drive motor **1014** that is located on the top shell **1006**.

FIG. **29** is a side cross-section of the storage system **1000** and illustrates the system cavity **1008** in greater detail. The storage system **1000** may also include a reaction component tray (or reaction component storage unit) **1020** and a fluid removal assembly **1022** that includes an elevator mechanism **1024**. The tray **1020** is configured to hold a plurality of tubes or containers for storing fluids. The elevator mechanism **1024** includes the drive motor **1014** and is configured to move components of the removal assembly **1022** bi-directionally along the Z-axis. In FIG. **29**, the tray **1020** is located in a fluid-removal position such that fluid held by the tray **1020** may be removed and delivered to, for example, a fluidic device for performing a desired reaction or for flushing the flow channels of the fluidic device.

Also shown, the temperature-control assembly **1012** may project into the system cavity **1008**. The temperature-control assembly **1012** is configured to control or regulate a temperature within the system cavity **1008**. In the illustrated embodiment, the temperature-control assembly **1012** includes a thermo-electric cooling (TEC) assembly.

FIG. **30** is a perspective view of the removal assembly **1022**. As shown, the removal assembly **1022** may include a pair of opposing guide rails **1032** and **1034**. The opposing guide rails **1032** and **1034** are configured to receive and direct the tray **1020** to the fluid-removal position shown in FIG. **29**. The guide rails **1032** and **1034** may include projected features or ridges **1035** that extend longitudinally along the guide rails **1032** and **1034**. The guide rails **1032** and **1034** are configured to be secured to the base shell **1004** (FIG. **28**). The removal assembly **1022** also includes support beams (or uprights) **1036** and **1038** that extend in a direction along the Z-axis. A guide plate **1040** of the removal assembly may be coupled to the support beams **1036** and **1038** at an elevated distance  $D_z$  and project therefrom along the XY-plane. In the illustrated embodiment, the guide plate **1040** is affixed to the support beams **1036** and **1038**.

The elevator mechanism **1024** includes structural supports **1041** and **1042**, a lead screw **1044** that extends between the structural supports **1041** and **1042**, and a stage assembly **1046** that includes a transport platform **1048**. The structural supports **1041** and **1042** are secured to opposite ends of the support beams **1036** and **1038** and are configured to support the elevator mechanism **1024** during operation. Threads of the lead screw **1044** are operatively coupled to the stage assembly **1046** such that when the lead screw **1044** is rotated,

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the stage assembly **1046** moves in a linear direction along the Z-axis (indicated by the double arrows).

The transport platform **1048** is configured to hold an array of sipper tubes **1050**. The sipper tubes **1050** may be in fluid communication with a system pump (not shown) that is configured to direct a flow of fluid through the sipper tubes **1050**. As shown, the sipper tubes **1050** include distal portions **1052** that are configured to be inserted into component wells **1060** (shown in FIG. **31**) of the tray **1020**. The distal portions **1052** extend through corresponding openings **1053** of the guide plate **1040**.

The elevator mechanism **1024** is configured to move the sipper tubes **1050** between withdrawn and deposited levels. At the deposited level (shown in FIGS. **50** and **51**), the distal portions **1052** of the sipper tubes **1050** are inserted into the component wells **1060** to remove fluid therefrom. At the withdrawn level, the distal portions **1052** are completely removed from the tray **1020** such that the tray **1020** may be removed from the system cavity **1008** (FIG. **28**) without damage to the sipper tubes **1050** or the tray **1020**. More specifically, when the drive motor **1014** rotates the lead screw **1044**, the stage assembly **1046** moves along the Z-axis in a direction that is determined by a rotational direction of the lead screw **1044**. Consequently, the transport platform **1048** moves along the Z-axis while holding the sipper tubes **1050**. If the transport platform **1048** advances toward the guide plate **1040**, the distal portions **1052** slide through the corresponding openings **1053** of the guide plate **1040** toward the tray **1020**. The guide plate **1040** is configured to prevent distal portions **1052** from becoming misaligned with the component wells **1060** before the distal portions **1052** are inserted therein. When the elevator mechanism **1024** moves the stage assembly **1046** away from the guide plate **1040**, a distance ( $\Delta Z$ ) between the transport platform **1048** and the guide plate **1040** increases until the distal portions **1052** are withdrawn from the component wells **1060** of the tray **1020**.

FIG. **30** illustrates additional features for operating the elevator mechanism **1024**. For example, the stage assembly **1046** may also include a guide pin **1058** (also shown in FIG. **29**) that is affixed to and extends from the transport platform **1048** in a direction that is parallel to the sipper tubes **1050**. The guide pin **1058** also extends through a corresponding opening **1053** of the guide plate **1040**. In the illustrated embodiment, the guide pin **1058** extends a greater distance than the sipper tubes **1050** so that the guide pin **1058** reaches the tray **1020** before the sipper tubes **1050** are inserted into the component wells **1060**. Thus, if the tray **1020** is misaligned with respect to the sipper tubes **1050**, the guide pin **1058** may engage the tray **1020** and adjust the position of the tray **1020** so that the component wells **1060** are properly aligned with the corresponding sipper tubes **1050** before the sipper tubes **1050** are inserted therein.

In addition to the above, the removal assembly **1022** may include a position sensor **1062** and a location sensor (not shown). The position sensor **1062** is configured to receive a flag **1063** (shown in FIG. **34**) of the tray **1020** to determine that the tray **1020** is present in the system cavity **1008** (FIG. **28**) and at least approximately aligned for receiving the sipper tubes **1050**. The location sensor may detect a flag **1064** of the stage assembly **1046** to determine a level of the stage assembly **1046**. If the flag **1064** has not reached a threshold level along the Z-axis, the location sensor may communicate with the workstation **160** (or other assay system) to notify the user that the tray **1020** is not ready for removal. The workstation **160** could also prevent the user from opening the system door **1010**.



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Furthermore, when the distal portions **1052** of the sipper tubes **1050** are initially inserted into the component wells **1060**, the sipper tubes **1050** may pierce protective foils that cover the component wells **1060**. In some instances, the foils may grip the sipper tubes **1050**. When the sipper tubes **1050** are subsequently withdrawn from the corresponding component wells **1060**, the gripping of the protective foils may collectively lift the tray **1020**. However, in the illustrated embodiment, the ridges **1035** are configured to grip a tray base **1070** (FIG. **31**) and prevent the tray base **1070** from being lifted in a direction along the Z-axis. For example, the ridges **1035** may grip a lip **1071** of the tray base **1070**.

FIGS. **31-34** illustrate different views of the tray **1020**. The tray **1020** is configured to hold a plurality of component wells **1060**. The component wells **1060** may include various reaction components, such as, but not limited to, one or more samples, polymerases, primers, denaturants, linearization mixes for linearizing DNA, enzymes suitable for a particular assay (e.g., cluster amplification or SBS), nucleotides, cleavage mixes, oxidizing protectants, and other reagents. In some embodiments, the tray **1020** may hold all fluids that are necessary to perform a predetermined assay. In particular embodiments, the tray **1020** may hold all reaction components necessary for generating a sample (e.g., DNA clusters) within a flow cell and performing sample analysis (e.g., SBS). The assay may be performed without removing or replacing any of the component wells **1060**.

The component wells **1060** include rectangular component wells **1060A** (shown in FIGS. **35-36**) and tubular component wells **1060B** (shown in FIG. **37**). The tray **1020** includes a tray base **1070** and a tray cover **1072** coupled to the tray base **1070**. As shown in FIGS. **31** and **32**, the tray cover **1072** includes a handle **1074** that is sized and shaped to be gripped by a user of the tray **1020**. The tray cover **1072** may also include a grip recess **1076** that is sized and shaped to receive one or more fingers of the user.

As shown in FIGS. **31** and **32**, the tray cover **1072** may include a plurality of tube openings **1080** that are aligned with corresponding component wells **1060**. The tube openings **1080** may be shaped to direct the sipper tubes **1050** (exemplary sipper tubes **1050** are shown in FIG. **31**) into the corresponding component wells **1060**. As shown in FIG. **32**, the tray cover **1072** also includes a pin opening **1082** that is sized and shaped to receive the guide pin **1058**. The guide pin **1058** is configured to provide minor adjustments to the position of the tray **1020** if the guide pin **1058** approaches and enters the pin opening **1082** in a misaligned manner. Also shown, the tray **1020** may include an identification tag **1084** along a surface of the tray cover **1072**. The identification tag **1084** is configured to be detected by a reader to provide the user with information regarding the fluids held by the component wells **1060**.

As shown in FIGS. **33** and **34**, the tube openings **1080** are at least partially defined by rims **1086** that project from a surface **1073** of the tray cover **1072**. The rims **1086** project a small distance away from the surface **1073** to prevent inadvertent mixing of fluids that are accidentally deposited onto the tray cover **1072**. Likewise, the identification tag **1084** may be attached to a raised portion **1088** of the tray cover **1072**. The raised portion **1088** may also protect the identification tag **1084** from inadvertently contacting fluids.

FIG. **35** shows a side cross-sectional view of the component well **1060A**, and FIG. **36** shows a bottom perspective view of the component well **1060A**. As shown, the component well **1060A** includes opposite first and second ends **1091** and **1092** and a reservoir **1090** (FIG. **35**) extending therebetween. The reservoir **1090** has a depth  $D_R$  (FIG. **35**) that

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increases as the reservoir **1090** extends from the second end **1092** to the first end **1091**. The component well **1060A** is configured to receive the sipper tube **1050** in a deeper portion of the reservoir **1090**. As shown in FIG. **36**, the component well **1060A** includes a plurality of projections **1094** along an exterior surface that are configured to rest upon a surface of the tray base **1070**.

FIG. **37** is a perspective view of the component well **1060B**. As shown, the component well **1060B** may also include a plurality of projections **1096** around an exterior surface of the component well **1060B**. The component well **1060B** extends along a longitudinal axis **1097** and has a profile that tapers as the component well **1060B** extends longitudinally to a bottom **1098**. The bottom **1098** may have a substantially planar surface.

FIG. **61** illustrates a method **960** for performing an assay for biological or chemical analysis. In some embodiments, the assay may include a sample generation protocol and a sample analysis protocol. For example, the sample generation protocol may include generating clusters of DNA through bridge amplification and the sample analysis protocol may include sequencing-by-synthesis (SBS) analysis using the clusters of DNA. The sample generation and sample analysis operations may be conducted within a common assay system, such as the assay system **100** or the workstation **160**, and without user intervention between the operations. For instance, a user may be able to load a fluidic device into the assay system. The assay system may automatically generate a sample for analysis and carry out the steps for performing the analysis.

With respect to FIG. **61**, the method **960** includes establishing at **962** a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components. The reaction components may be configured for conducting one or more assays. The fluidic device may be, for example, the fluidic device **300** or the flow cell **200** described above. In some embodiments, the sample area includes a plurality of reaction components (e.g., primers) immobilized thereon. The storage unit may be, for example, the storage unit **1020** described above. The reaction components may include sample-generation components that are configured to be used to generate the sample, and sample-analysis components that are configured to be used to analyze the sample. In particular embodiments, the sample-generation components include reaction components for performing bridge amplification as described above. Furthermore, in particular embodiments, the sample-analysis components include reaction components for performing SBS analysis as described above.

The method **960** also includes generating at **964** a sample at the sample area of the fluidic device. The generating operation **964** may include flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. For example, a thermocycler may be used to facilitate hybridizing nucleic acids. However, isothermal methods can be used if desired. Furthermore, a flow rate of the fluids may be controlled to permit hybridization or other desired chemical reactions. In particular embodiments, the generating operation **964** includes conducting multiple bridge-amplification cycles to generate a cluster of DNA.

An exemplary protocol for bridge amplification can include the following steps. A flow cell is placed in fluid communication with a reaction component storage unit. The flow cell includes one or more surfaces to which are attached pairs of primers. A solution having a mixture of target nucleic acids of different sequences is contacted with a solid support.



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The target nucleic acids can have common priming sites that are complementary to the pairs of primers on the flow cell surface such that the target nucleic acids bind to a first primer of the pairs of primers on the flow cell surface. An extension solution containing polymerase and nucleotides can be introduced to the flow cell such that a first amplification product, which is complementary to the target nucleic acid, is formed by extension of the first primer. The extension solution can be removed and replaced with a denaturation solution. The denaturation solution can include chemical denaturants such as sodium hydroxide and/or formamide. The resulting denaturation conditions release the original strand of the target nucleic acid, which can then be removed from the flow cell by removing the denaturation solution and replacing it with the extension solution. In the presence of the extension solution the first amplification product, which is attached to the support, can then hybridize with a second primer of the primer pairs attached to the flow cell surface and a second amplification product comprising an attached nucleic acid sequence complementary to the first amplification product can be formed by extension of the second primer. Repeated delivery of the denaturation solution and extension solution can be used to form clusters of the target nucleic acid at discrete locations on the surface of the flow cell. Although the above protocol is exemplified using chemical denaturation, it will be understood that thermal denaturation can be carried out instead albeit with similar primers and target nucleic acids. Further description of amplification methods that can be used to produce clusters of immobilized nucleic acid molecules is provided, for example, in U.S. Pat. No. 7,115,400; U.S. Publication No. 2005/0100900; WO 00/18957; or WO 98/44151, each of which is incorporated by reference herein.

The method **960** also includes analyzing at **966** the sample at the sample area. Generally, the analyzing operation **966** may include detecting any detectable characteristic at the sample area. In particular embodiments, the analyzing operation **966** includes flowing at least one sample-analysis component to the sample area. The sample-analysis component may react with the sample to provide optically detectable signals that are indicative of an event-of-interest (or desired reaction). For example, the sample-analysis components may be fluorescently-labeled nucleotides used during SBS analysis. When excitation light is incident upon the sample having fluorescently-labeled nucleotides incorporated therein, the nucleotides may emit optical signals that are indicative of the type of nucleotide (A, G, C, or T), and the imaging system may detect the optical signals.

A particularly useful SBS protocol exploits modified nucleotides having removable 3' blocks, for example, as described in WO 04/018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. Repeated cycles of SBS reagents can be delivered to a flow cell having target nucleic acids attached thereto, for example, as a result of the bridge amplification protocol set forth above. The nucleic acid clusters can be converted to single stranded form using a linearization solution. The linearization solution can contain, for example, a restriction endonuclease capable of cleaving one strand of each cluster. Other methods of cleavage can be used as an alternative to restriction enzymes or nicking enzymes, including inter alia chemical cleavage (e.g., cleavage of a diol linkage with periodate), cleavage of abasic sites by cleavage with endonuclease (for example 'USER', as supplied by NEB, Ipswich, Mass., USA, part number M5505S), by exposure to heat or alkali, cleavage of ribonucleotides incorporated into amplification products otherwise comprised of deoxyribonucleotides, photochemical cleavage or cleavage of a peptide linker. After the

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linearization step a sequencing primer can be delivered to the flow cell under conditions for hybridization of the sequencing primer to the target nucleic acids that are to be sequenced.

The flow cell can then be contacted with an SBS extension reagent having modified nucleotides with removable 3' blocks and fluorescent labels under conditions to extend a primer hybridized to each target nucleic acid by a single nucleotide addition. Only a single nucleotide is added to each primer because once the modified nucleotide has been incorporated into the growing polynucleotide chain complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase cannot add further nucleotides. The SBS extension reagent can be removed and replaced with scan reagent containing components that protect the sample under excitation with radiation. Exemplary components for scan reagent are described in US publication US 2008/0280773 A1 and U.S. Ser. No. 13/018,255, each of which is incorporated herein by reference. The extended nucleic acids can then be fluorescently detected in the presence of scan reagent. Once the fluorescence has been detected, the 3' block may be removed using a deblock reagent that is appropriate to the blocking group used. Exemplary deblock reagents that are useful for respective blocking groups are described in WO04018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. The deblock reagent can be washed away leaving target nucleic acids hybridized to extended primers having 3' OH groups that are now competent for addition of a further nucleotide. Accordingly the cycles of adding extension reagent, scan reagent, and deblock reagent, with optional washes between one or more of the steps, can be repeated until a desired sequence is obtained. The above cycles can be carried out using a single extension reagent delivery step per cycle when each of the modified nucleotides has a different label attached thereto, known to correspond to the particular base. The different labels facilitate discrimination between the bases added during each incorporation step. Alternatively, each cycle can include separate steps of extension reagent delivery followed by separate steps of scan reagent delivery and detection, in which case two or more of the nucleotides can have the same label and can be distinguished based on the known order of delivery.

Continuing with the example of nucleic acid clusters in a flow cell, the nucleic acids can be further treated to obtain a second read from the opposite end in a method known as paired end sequencing. Methodology for paired end sequencing are described in PCT publication WO07010252, PCT application Serial No. PCTGB2007/003798 and US patent application publication US 2009/0088327, each of which is incorporated by reference herein. In one example, a series of steps may be performed as follows; generate clusters as set forth above, linearize as set forth above, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above, "invert" the target nucleic acids on the flow cell surface by synthesizing a complementary copy, linearize the resynthesized strand, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above. The inversion step can be carried out by delivering reagents as set forth above for a single cycle of bridge amplification.

Although the analyzing operation has been exemplified above with respect to a particular SBS protocol, it will be understood that other protocols for sequencing any of a variety of other molecular analyses can be carried out as desired. Appropriate modification of the apparatus and methods to



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accommodate various analyses will be apparent in view of the teaching set forth herein and that which is known about the particular analysis method.

In some embodiments, the method **960** is configured to be conducted with minimal user intervention. The generating and analyzing operations **964** and **966** may be conducted in an automated manner by an assay system. For example, in some cases, a user may only load the fluidic device and the storage unit and activate the assay system to perform the method **960**. In some embodiments, during the generating and analyzing operations **964** and **966**, the storage unit and the fluidic device remain in fluid communication from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. In other words, the fluidic device and the storage unit may remain in fluid communication from before the sample is generated until after the sample is analyzed. In some embodiments, the fluidic device is continuously held by the device holder from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. During such time, the device holder and an imaging lens may be automatically moved with respect to each other. The storage unit and the fluidic device may remain in fluid communication when the fluidic device and the imaging lens are automatically moved with respect to each other. In some embodiments, the assay system is contained within a workstation housing and the generating and analyzing operations **964** and **966** are conducted exclusively within the workstation housing.

FIG. **38** is a schematic illustration of an optical imaging system **600** formed in accordance with one embodiment. The imaging system **600** includes an optical assembly **602**, a light source (or excitation light) module or assembly **604**, a flow cell **606** having a sample area **608**, and imaging detectors **610** and **612**. The light source module **604** includes first and second excitation light sources **614** and **616** that are configured to illuminate the sample area **608** with different excitation spectra. In particular embodiments, the first and second excitation light sources **614** and **616** comprise first and second semiconductor light sources (SLSs). SLSs may include light-emitting diodes (LEDs) or laser diodes. However, other light sources may be used in other embodiments, such as lasers or arc lamps. The first and second SLSs may have fixed positions with respect to the optical assembly **602**.

As shown, the optical assembly **602** may include a plurality of optical components. For example, the optical assembly **602** may include lenses **621-627**, emission filters **631-634**, excitation filters **635** and **636**, and mirrors **641-645**. The plurality of optical components are arranged to at least one of (a) direct the excitation light toward the sample area **608** of the flow cell **606** or (b) collect emission light from the sample area **608**. Also shown, the imaging system **600** may also include a flow system **652** that is in fluid communication with the flow cell **606** and a system controller **654** that is communicatively coupled to the first and second excitation light sources **614** and **616** and the flow system **652**. The controller **654** is configured to activate the flow system **652** to flow reagents to the sample area **608** and activate the first and second SLSs after a predetermined time period.

For example, FIG. **60** illustrates a method **900** for performing an assay for biological or chemical analysis. In particular embodiments, the assay may include a sequencing-by-synthesis (SBS) protocol. The method **900** includes flowing reagents through a flow channel of a flow cell at **902**. The flow cell may have a sample area that includes a sample with biomolecules configured to chemically react with the reagents. The method **900** also includes illuminating the sample area at **904** with first and second semiconductor light

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sources (SLSs). The first and second SLSs provide first and second excitation spectra, respectively. The biomolecules of the sample may provide light emissions that are indicative of a binding reaction when illuminated by the first or second SLSs. Furthermore, the method **900** includes detecting the light emissions from the sample area at **906**. Optionally, the method **900** may include moving the flow cell at **908** relative to an imaging lens and repeating the illuminating and detecting operations **904** and **906**. The steps shown in FIG. **60** and exemplified above can be repeated for multiple cycles of a sequencing method.

FIGS. **39** and **40** illustrate various features of a motion-control system **700** formed in accordance with one embodiment that may be used with the imaging system **600**. The motion-control system **700** includes an optical base plate **702** and a sample deck **708** that is movably coupled to the base plate **702**. As shown, the base plate **702** has a support side **704** and a bottom side **705**. The support and bottom sides **704** and **705** face in opposite directions along the Z-axis. The base plate **702** is configured to support a majority of the optical components of the optical assembly **602** (FIG. **38**) on the support side **704**. The base plate **702** and the sample deck **708** may be movably coupled to each other by an intermediate support **715** and a face plate **722** such that the sample holder **650** may substantially rotate about the X and Y axes, shift along the Y axis, and slide along the X axis.

FIG. **40** is an isolated perspective view of the intermediate support **715**, a motor assembly **724**, and a movable platform **726** of the sample deck **708** (FIG. **39**). The motor assembly **724** is operatively coupled to the platform **726** and is configured to slide the platform **726** bi-directionally along the X-axis. As shown, the intermediate support **715** includes a tail end **728** and an imaging end **730**. The intermediate support **715** may include pins **746** and **748** proximate to the imaging end **730** that project away from each other along the Y-axis. Proximate to the imaging end **730**, the intermediate support **715** may include a lens opening **750** that is sized and shaped to allow the imaging lens **623** (FIG. **38**) to extend therethrough. In the illustrated embodiment, the pins **746** and **748** have a common line **755** extending therethrough that also extends through the lens opening **750**.

Returning to FIG. **39**, the platform **726** is coupled to the bottom side **705** through the intermediate support **715**. Accordingly, a weight of the sample deck **708** may be supported by the base plate **702**. Furthermore, the motion-control system **700** may include a plurality of alignment devices **733**, **735**, **737**, and **739** that are configured to position the sample holder **650**. In the illustrated embodiment, the alignment devices **733**, **735**, **737**, and **739** are micrometers. The alignment device **733** is operatively coupled to the tail end **728** of the intermediate support **715**. When the alignment device **733** is activated, the tail end **728** may be moved in a direction along the Z-axis. Consequently, the intermediate support **715** may rotate about the pins **746** and **748** (FIG. **40**) or, more specifically, about the line **755**. When the alignment devices **735** and **737** are activated, the sample holder **650** may shift along the Y-axis as directed. When the alignment device **739** is activated, the sample holder **650** may rotate about an axis of rotation  $R_7$  that extends parallel to the X-axis.

FIGS. **41-42** show a perspective view and plan view, respectively, of the optical base plate **702** that may be used with the imaging system **600** (FIG. **38**). In some embodiments of the imaging system **600**, one or more of the optical components **621-627**, **631-636**, and **641-645** (FIG. **38**) can have a fixed position in the optical assembly **602** such that the fixed (or static) optical component does not move during operation of the imaging system **600**. For example, the base



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plate **702** is configured to support a plurality of optical components and other parts of the imaging system **600**. As shown, the base plate **702** constitutes a substantially unitary structure having a support side (or surface) **704** that faces in a direction along the Z-axis. In the illustrated embodiment, the support side **704** is not continuously smooth, but may have various platforms **716-718**, depressions (or receiving spaces) **719-721**, and component-receiving spaces **711-714** that are located to arrange the optical assembly **602** in a predetermined configuration. As shown in FIG. **42**, each of the component-receiving spaces **711-714** has respective reference surfaces **781-784**. In some embodiments, the reference surfaces **781-784** can facilitate orienting and holding corresponding optical components in desired positions.

FIGS. **43** and **44** show a front perspective view and a cutaway rear perspective view, respectively, of an optical device **732**. As shown in FIG. **43**, the optical device **732** is oriented relative to mutually perpendicular axes **791-793**. The axis **791** may extend along a gravitational force direction and/or parallel to the Z-axis illustrated above. In particular embodiments, the optical device **732** is configured to be positioned within the component-receiving space **713** (FIG. **43**) of the base plate **702** (only a portion of the base plate **702** is shown in FIGS. **43** and **44**).

The component-receiving space **713** has one or more surfaces that define an accessible spatial region where an optical component may be held. These one or more surfaces may include the reference surface(s) described below. In the illustrated embodiment, the component-receiving space **713** is a component cavity of the base plate **701** that extends a depth within the base plate **702**. However, the base plate **702** may form the component-receiving space in other manners. For example, in a similar way that the base plate **702** may form a cavity, the base plate **702** may also have one or more raised platforms including surfaces that surround and define the component-receiving space. Accordingly, the base plate **702** may be shaped to partially or exclusively provide the component-receiving space. The base plate **702** may include the reference surface. In alternative embodiments, sidewalls may be mounted on the base plate **702** and configured to define the spatial region. Furthermore, other optical devices mounted to the base plate **702** may define the component-receiving spaces. As used herein, when an element “defines” a component-receiving space, the element may exclusively define the component-receiving space or may only partially define the component-receiving space.

The optical device **732** can be removably mounted to the base plate **702** in the component-receiving space **713**, but may be configured to remain in a fixed position during operation of the imaging system. However, in alternative embodiment, the optical device **732** is not positioned within the component-receiving space **713**, but may be positioned elsewhere, such as on a platform of the support side **704**. In the illustrated embodiment, the optical device **732** includes a mounting device **734** and an optical component **736** that is configured to reflect and/or transmit light therethrough. The mounting device **734** is configured to facilitate holding the optical component **736** in a desired orientation and also removably mount the optical component **736** to the base plate **702**. The mounting device **734** includes a component retainer **738** and a biasing element **740** that is operatively coupled to the retainer **738**.

In the illustrated embodiment, the optical component **736** comprises an optical filter that transmits optical signals therethrough while filtering for a predetermined spectrum. However, other optical components may be used in alternative embodiments, such as lenses or mirrors. As shown, the optical

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component **736** may include optical surfaces **742** and **744** that face in opposite directions and define a thickness  $T_3$  of the optical component **736** therebetween. As shown, the optical surfaces **742** and **744** may be continuously smooth and planar surfaces that extend parallel to each other such that the thickness  $T_3$  is substantially uniform. However, the optical surfaces **742** and **744** may have other contours in alternative embodiments. The optical component **736** may have a plurality of component edges **751-754** (FIG. **43**) that define a perimeter or periphery. The periphery surrounds the optical surfaces **742** and **744**. As shown, the periphery is substantially rectangular, but other geometries may be used in alternative embodiments (e.g., circular).

The retainer **738** facilitates holding the optical component **736** in a desired orientation. In the illustrated embodiment, the retainer **738** is configured to engage the optical surface **742** and extend around at least a portion of the periphery to retain the optical component **736**. For example, the retainer **738** may include a wall portion **756** and a frame extension **758** that extends from the wall portion **756** along the periphery of the optical component **736** (e.g., the component edge **752** (FIG. **43**)). In the illustrated embodiment, the frame extension **758** may form a bracket that limits movement of the optical component **736**. More specifically, the frame extension **758** may include a proximal arm **760** and a distal arm **762**. The proximal arm **760** extends from the wall portion **756** along the component edge **752** and the axis **791**. The distal arm **762** extends from the proximal arm **760** along the component edge **751**. The distal arm **762** includes a projection or feature **764** that extends toward and engages the optical component **736**. Also shown, the retainer **738** may include a grip member **766** that is located opposite the frame extension **758**. The grip member **766** and the frame extension **758** may cooperate in limiting movement of the optical component **736** along the axis **793**. The retainer **738** may grip a portion of the periphery of the optical component **736**.

As shown in FIGS. **43** and **44**, the wall portion **756** is configured to engage the optical surface **742**. For example, the wall portion **756** has a mating surface **770** (FIG. **43**) that faces the optical component **736**. In some embodiments, the wall portion **756** includes a plurality of orientation features **771-773** (FIG. **43**) along the mating surface **770**. The orientation features **771-773** are configured to directly engage the optical surface **742** of the optical component **736**. When the orientation features **771-773** directly engage the optical surface **742**, the optical surface **742** (and consequently the optical component **736**) is positioned in a desired orientation with respect to the retainer **738**. As shown in FIG. **43**, the reference surface **783** of the component-receiving space **713** also includes a plurality of orientation features **761-763**. The orientation features **761-763** are configured to directly engage the optical surface **744**. Furthermore, the orientation features **761-763** may be arranged such that each of the orientation features **761-763** generally opposes a corresponding one of the orientation features **771-773**.

Also shown in FIG. **44**, the wall portion **756** has a non-mating surface **774** that faces in an opposite direction with respect to the mating surface **770** (FIG. **43**). The wall portion **756** includes an element projection **776** that extends away from the non-mating surface **774** and the optical component **736**. The biasing element **740** is configured to couple to the element projection **776**. In the illustrated embodiment, the element projection **776** and the biasing element **740** extend into a slot **778** of the component-receiving space **713**. The slot **778** is sized and shaped to receive the biasing element **740**. The slot **778** has an element surface **780** that engages the biasing element **740**.



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FIG. 45 shows an isolated front view of the optical device 732, and FIG. 46 shows how the optical device 732 may be removably mounted to the base plate 702. To removably mount the optical component 736, the optical component 736 may be positioned within a component-receiving space 789 of the mounting device 734 that is generally defined by the wall portion 756 (FIG. 46), the frame extension 758, and the grip member 766. In particular embodiments, when the optical component 736 is positioned within the mounting device 734, the optical component 736 is freely held within the component-receiving space 789. For instance, the optical component 736 may not form an interference fit with the retainer 738. Instead, during a mounting operation, the optical component 736 may be held within the component-receiving space 789 by the wall portion 756, the frame extension 758, the grip member 766 and, for example, an individual's hand. However, in alternative embodiments, the optical component 736 may form an interference fit with the retainer 738 or may be confined within a space that is defined only by the retainer 738.

With respect to FIG. 46, during the mounting operation, the biasing element 740 may be initially compressed so that the mounting device 734 may clear and be inserted into the component-receiving space 713. For example, the biasing element 740 may be compressed by an individual's finger to reduce the size of the optical device 732, or the biasing element 740 may be compressed by first pressing the biasing element 740 against the element surface 780 and then advancing the retainer 738 into the component-receiving space 713. Once the optical device 732 is placed within the component-receiving space 713, the stored mechanical energy of the compressed biasing element 740 may move the retainer 738 and the optical component 736 toward the reference surface 783 until the optical surface 744 directly engages the reference surface 783. More specifically, the optical surface 744 may directly engage the orientation features 761-763 (FIG. 43) of the reference surface 783. As shown in FIG. 46, when the optical component 736 is mounted, a small gap  $G_1$  may exist between the optical surface 742 and the mating surface 770 (FIG. 43) because of the orientation features 771-773 (FIG. 43), and a small gap  $G_2$  may exist between the optical surface 744 and the reference surface 783 because of the orientation features 761-763 (FIG. 43).

In the mounted position, the biasing element 740 provides an alignment force  $F_A$  that holds the optical surface 744 against the reference surface 783. The optical and reference surfaces 744 and 783 may be configured to position the optical component 736 in a predetermined orientation. The alignment force  $F_A$  is sufficient to hold the optical component 736 in the predetermined orientation throughout operation of the imaging system. In other words, the mounting device 734 and the reference surface 783 may prevent the optical component 736 from moving in a direction along the axis 792. Furthermore, in the mounted position, the projection 764 (FIG. 43) may press against the component edge 751 (FIG. 43) to prevent the optical component 736 from moving in a direction along the axis 791. The frame extension 758 and the grip member 766 may prevent or limit movement of the optical component 736 in a direction along the axis 793. Accordingly, the component-receiving space 713 and the mounting device 734 may be configured with respect to each other to hold the optical component 736 in a predetermined orientation during imaging sessions.

As shown in FIG. 45, when the optical component 736 is in the mounted position, a space portion 798 of the optical surface 744 may face and interface with the reference surface 783, and a path portion 799 of the optical surface 744 may

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extend beyond the support side 704 into an optical path taken by optical signals. Also shown in FIG. 46, the component-receiving space 713 may extend a depth  $D_C$  into the base plate 702 from the support side 704.

The biasing element 740 may comprise any elastic member capable of storing mechanical energy to provide the alignment force  $F_A$ . In the illustrated embodiment, the elastic member comprises a coil spring that pushes the optical surface 744 against the reference surface 783 when compressed. However, in alternative embodiments, the elastic member and the component-receiving space may be configured such that the elastic member pulls the optical surface against the reference surface when extended. For example, a coil spring may have opposite ends in which one end is attached to the element surface in a slot that extends from the reference surface and another end is attached to the retainer. When the coil spring is extended, the coil spring may provide an alignment force that pulls the optical component against the reference surface. In this alternative embodiment, a rubber band may also be used.

In alternative embodiments, the mounting device 734 may be used to affix the optical component 736 to the base plate 702 using an adhesive. More specifically, the optical component 736 may be held against the reference surface 783 by the mounting device 734. An adhesive may be deposited into the gap  $G_2$  between the optical surface 744 and the reference surface 783. After the adhesive cures, the mounting device 734 may be removed while the optical component 736 remains affixed to the reference surface 783 by the adhesive.

FIG. 47 is a block diagram illustrating a method 800 of assembling an optical train. The method 800 includes providing an optical base plate at 802 that has a component-receiving space. The base plate and the component-receiving space may be similar to the base plate 702 and the component-receiving space 713 described above. The method 800 also includes inserting an optical component at 804 into the component-receiving space. The optical component may be similar to the optical component 736 described above and include an optical surface that is configured to reflect or transmit light therethrough. The optical surface may have a space portion that faces a reference surface of the component-receiving space and a path portion that extends beyond the support side into an optical path. The method 800 also includes providing an alignment force at 806 that holds the optical surface against the reference surface to orient the optical component. The optical and reference surfaces may be configured to hold the optical component in a predetermined orientation when the alignment force is provided. In some embodiments, the method 800 may also include removing the optical component at 808 and, optionally, inserting a different optical component at 810 into the component-receiving space. The different optical component may have the same or different optical qualities. In other words, the different optical component may be a replacement that has the same optical qualities or the different optical component may have different optical qualities.

FIGS. 48 and 49 provide a perspective view and a side view, respectively, of the light source (or excitation light module) 604. As used herein, a light source module includes one or more light sources (e.g., lasers, arc lamps, LEDs, laser diodes) that are secured to a module frame and also includes one or more optical components (e.g., lenses or filters) that are secured to the module frame in a fixed and predetermined position with respect to said one or more light sources. The light source modules may be configured to be removably coupled within an imaging system so that a user may relatively quickly install or replace the light source module. In particular embodiments, the light source module 604 consti-



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tutes a SLS module **604** that includes the first and second SLSs **614** and **616**. As shown, the SLS module **604** includes a module frame **660** and a module cover **662**. A plurality of imaging components may be secured to the module frame **660** in fixed positions with respect to each other. For example, the first and second SLSs **614** and **616**, the excitation filter **635**, and the lenses **624** and **625** may be mounted onto the module frame **660**. In addition, the SLS module **604** may include first and second heat sinks **664** (FIG. **48**) and **666** that are configured to transfer thermal energy from the first and second SLSs **614** and **616**, respectively.

The SLS module **604** and the module frame **660** may be sized and shaped such that an individual could hold the SLS module **604** with the individual's hands and readily manipulate for installing into the imaging system **600**. As such, the SLS module **604** has a weight that an adult individual could support.

The SLS module **604** is configured to be placed within the module-receiving space **719** (FIG. **41**) and removably coupled to the base plate **702** (FIG. **41**). As shown, the module frame **660** has a plurality of sides including a mounting side **670** and an engagement face **671** (FIG. **48**). In the illustrated embodiment, the module frame **660** is substantially rectangular or block-shaped, but the module frame **660** may have other shapes in alternative embodiments. The mounting side **670** is configured to be mounted to the base plate **702** within the module-receiving space **719**. As such, at least a portion of the module-receiving space **719** may be shaped to receive and hold the SLS module **604**. Similar to the component-receiving space **713**, the module-receiving space **719** may be defined by one or more surfaces that provide an accessible spatial region where the SLS module **604** may be held. The surface(s) may be of the base plate **702**. For example, in the illustrated embodiment, the module-receiving space **719** is a depression of the base plate **702**. The mounting side **670** may have a contour that substantially complements the base plate **702** and, more specifically, the module-receiving space **719**. For example, the mounting side **670** may be substantially planar and include a guidance pin **672** (FIG. **49**) projecting therefrom that is configured to be inserted into a corresponding hole (not shown) in the base plate **702**. The guidance pin **672** may be a fastener (e.g., screw) configured to facilitate removably coupling the module frame **660** to the base plate **702**. In particular embodiments, the guidance pin **672** is inserted into the base plate **702** at a non-orthogonal angle. As shown in FIG. **49**, the heat sink **666** may be coupled to the module frame **660** such that an offset **676** exists from the mounting side **670** to the heat sink **666**.

The module frame **660** may include first and second light passages **682** and **684** that intersect each other at a passage intersection **685**. The SLSs **614** and **616** may be secured to the module frame **660** and have fixed positions with respect to each other. The SLSs **614** and **616** are oriented such that optical signals are substantially directed along optical paths through the respective light passages **682** and **684** toward the passage intersection **685**. The optical paths may be directed toward the excitation filter **635**. In the illustrated embodiment, the optical paths are perpendicular to one another until reaching the excitation filter **635**. The excitation filter **635** is oriented to reflect at least a portion of the optical signals generated by the SLS **616** and transmit at least a portion of the optical signals generated by the SLS **614**. As shown, the optical signals from each of the SLSs **614** and **616** are directed along a common path and exit the SLS module **604** through a common module window **674**. The module window **674** extends through the engagement face **671**.

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FIG. **50** is a plan view of the SLS module **604** mounted onto the base plate **702**. In the illustrated embodiment, the SLS module **604** is configured to rest on the base plate **702** such that the gravitational force *g* facilitates holding the SLS module **604** thereon. As such, the SLS module **604** may provide an integrated device that is readily removed or separated from the optical assembly **600**. For example, after removing a housing (not shown) of the assay system or after receiving access to the optical assembly, the SLS module **604** may be grabbed by an individual and removed or replaced. When the SLS module **604** is located on the base plate **702**, the engagement face **671** may engage an optical device **680**. The optical device **680** may be adjacent to the module window **674** such that the optical signals generated by the SLS module **604** are transmitted through the optical device **680**.

Although the illustrated embodiment is described as using an SLS module with first and second SLSs, excitation light may be directed onto the sample in other manners. For example, the SLS module **604** may include only one SLS and another optical component (e.g., lens or filter) having fixed positions with respect to each other in a module frame. Likewise, more than two SLSs may be used. In a similar manner, light modules may include only one laser or more than two lasers.

However, embodiments described herein are not limited to only having modular excitation systems, such as the SLS module **604**. For example, the imaging system **600** may use a light source that is not mounted to a module frame. More specifically, a laser could be directly mounted to the base plate or other portion of the imaging system or may be mounted to a frame that, in turn, is mounted within the imaging system.

Returning to FIG. **38**, the imaging system **600** may have an image-focusing system **840** that includes the object or sample holder **650**, an optical train **842**, and the imaging detector **610**. The optical train **842** is configured to direct optical signals from the sample holder **650** (e.g., light emissions from the sample area **608** of the flow cell **606**) to a detector surface **844** of the imaging detector **610**. As shown in FIG. **38**, the optical train **842** includes the optical components **623**, **644**, **634**, **633**, **621**, **631**, and **642**. The optical train **842** may include other optical components. In the illustrated configuration, the optical train **842** has an object or sample plane **846** located proximate to the sample holder **650** and an image plane **848** located proximate to the detector surface **844**. The imaging detector **610** is configured to obtain object or sample images at the detector surface **844**.

In some embodiments, the image-focusing system **840** is configured to move the image plane **848** relative to the detector **610** and capture a test image. More specifically, the image plane **848** may be moved such that the image plane **848** extends in a non-parallel manner with respect to the detector surface **844** and intersects the detector surface **844**. A location of the intersection may be determined by analyzing the test image. The location may then be used to determine a degree-of-focus of the imaging system **600**. In particular embodiments, the image-focusing system **840** utilizes a rotatable mirror that is operatively coupled to an actuator for moving the rotatable mirror. However, the image-focusing system **840** may move other optical components that direct the optical signals to the detector surface **844**, or the image-focusing system **840** may move the detector **610**. In either case, the image plane **848** may be relatively moved with respect to the detector surface **844**. For example, the image-focusing system **840** may move a lens.

In particular embodiments, the imaging detector **610** is configured to obtain test images using a rotatable mirror **642**



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to determine a degree-of-focus of the imaging system 600. As a result of the determined degree-of-focus, the imaging system 600 may move the sample holder 650 so that the object or sample is located within the sample plane 846. For example, the sample holder 650 may be configured to move the sample area 608 in a z-direction a predetermined distance (as indicated by  $\Delta z$ ).

FIG. 51 is a plan view that illustrates several of the components in the image-focusing system 840. As shown, the image-focusing system 840 includes a rotatable mirror assembly 850 that includes the mirror 642, a mounting assembly 852 having the mirror 642 mounted thereon, and an actuator or rotation mechanism 854 that is configured to rotate the mounting assembly 852 and the mirror 642 about an axis of rotation  $R_6$ . The mirror 642 is configured to reflect optical signals 863 that are received from the sample area 608 (FIG. 38) toward the imaging detector 610 and onto the detector surface 844. In the illustrated embodiment, the mirror 642 reflects the optical signals 863 directly onto the detector surface 844 (i.e., there are no intervening optical components that redirect the optical signals 863). However, in alternative embodiments, there may be additional optical components that affect the propagation of the optical signals 863.

In the illustrated embodiment, the image-focusing system 840 also includes positive stops 860 and 862 that are configured to prevent the mirror 642 from rotating beyond predetermined rotational positions. The positive stops 860 and 862 have fixed positions with respect to the axis  $R_6$ . The mounting assembly 852 is configured to pivot about the axis  $R_6$  between the positive stops 860 and 862 depending upon whether sample images or test images are being obtained. Accordingly, the mirror 642 may be rotated between a test position (or orientation) and an imaging position (or orientation). By way of example only, the mirror 642 may be rotated from approximately  $5^\circ$  to approximately  $12^\circ$  about the axis  $R_6$  between the different rotational positions. In particular embodiments, the mirror 642 may be rotated approximately  $8^\circ$  about the axis  $R_6$ .

FIG. 52 is a perspective view of the mirror assembly 850. As shown, the mounting assembly 852 includes an interior frame 864 and a support bracket 866. The interior frame 864 is configured to couple to the mirror 642 and also to the support bracket 866. The interior frame 864 and the support bracket 866 may interact with each other and a plurality of set screws 868 to provide minor adjustments to the orientation of the mirror 642. As such, the mounting assembly 852 may constitute a gimbal mirror mount assembly. Also shown, the mounting assembly 852 is coupled to the rotation mechanism 854. In the illustrated embodiment, the rotation mechanism 854 comprises a direct drive motor. However, a variety of alternative rotation mechanisms may be used, such as direct current (DC) motors, solenoid drivers, linear actuators, piezoelectric motors, and the like. Also shown in FIG. 52, the positive stop 860 may have a fixed position with respect to the rotation mechanism 854 and the axis  $R_6$ .

As discussed above, the rotation mechanism 854 is configured to rotate or pivot the mirror 642 about the axis  $R_6$ . As shown in FIG. 52, the mirror 642 has a geometric center C that extends along the axis  $R_6$ . The geometric center C of the mirror 642 is offset with respect to the axis  $R_6$ . In some embodiments, the rotation mechanism 854 is configured to move the mirror 642 between the test position and imaging position in less than 500 milliseconds. In particular embodiments, the rotation mechanism 854 is configured to move the mirror 642 between the test position and imaging position in less than 250 milliseconds or less than 160 milliseconds.

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FIG. 53 is a schematic diagram of the mirror 642 in the imaging position. As shown, the optical signals 863 from the sample area 608 (FIG. 38) are reflected by the mirror 642 and directed toward the detector surface 844 of the imaging detector 610. Depending upon the configuration of the optical train 842 and the z-position of the sample holder 610, the sample area 608 may be sufficiently in-focus or not sufficiently in-focus (i.e., out-of-focus). FIG. 53 illustrates two image planes 848A and 848B. The image plane 848A substantially coincides with the detector surface 844 and, as such, the corresponding sample image has an acceptable or sufficient degree-of-focus. However, the image plane 848B is spaced apart from the detector surface 844. Accordingly, the sample image obtained when the image plane 848B is spaced apart from the detector surface 844 may not have a sufficient degree-of-focus.

FIGS. 54 and 55 illustrate sample images 870 and 872, respectively. The sample image 870 is the image detected by the imaging detector 610 when the image plane 848A coincides with the detector surface 844. The sample image 872 is the image detected by the imaging detector 610 when the image plane 848B does not coincide with the detector surface 844. (The sample images 870 and 872 include clusters of DNA that provide fluorescent light emissions when excited by predetermined excitation spectra.) As shown in FIGS. 54 and 55, the sample image 870 has an acceptable degree-of-focus in which each of the clusters along the sample image 870 is clearly defined, and the sample image 872 does not have an acceptable degree-of-focus in which each of the clusters is clearly defined.

FIG. 56 is a schematic diagram of the mirror 642 in the focusing position. As shown, the mirror 642 in the focusing position has been rotated about the axis  $R_6$  an angle  $\theta$ . Again, the optical signals 863 from the sample area 608 (FIG. 38) are reflected by the mirror 642 and directed toward the detector surface 844 of the imaging detector 610. However, the optical train 842 in FIG. 56 is arranged so that the image plane 848 has been moved with respect to the detector surface 844. More specifically, the image plane 848 does not extend parallel to the detector surface 844 and, instead, intersects the detector surface 844 at a plane intersection PI. While the mirror 642 is in the focusing position, the imaging system 600 may obtain a test image of the sample area 608. As shown in FIG. 56, the plane intersections PI may occur at different locations on the detector surface 844 depending upon the degree to which the sample area 608 is in-focus during an imaging session.

For example, FIGS. 57 and 58 illustrate test images 874 and 876, respectively. The test image 874 represents the image obtained when the sample area 608 is in-focus, and the test image 876 represents the image obtained when the optical train 842 is out-of-focus. As shown, the test image 874 has a focused region or location  $FL_1$  that is located a distance  $XD_1$  away from a reference edge 880, and the test image 876 has a focused region or location  $FL_2$  that is located a distance  $XD_2$  away from a reference edge 880. The focused locations  $FL_1$  and  $FL_2$  may be determined by an image analysis module 656 (FIG. 38).

To identify the focused locations  $FL_1$  and  $FL_2$  in the test images 874 and 876, the image analysis module 656 may determine the location of an optimal degree-of-focus in the corresponding test image. More specifically, the analysis module 656 may determine a focus score for different points along the x-dimension of the test images 874 and 876. The analysis module 656 may calculate the focus score at each point based on one or more image quality parameters. Examples of image quality parameters include image con-



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trast, spot size, image signal to noise ratio, and the mean-square-error between pixels within the image. By way of example, when calculating a focus score, the analysis module **656** may calculate a coefficient of variation in contrast within the image. The coefficient of variation in contrast represents an amount of variation between intensities of the pixels in an image or a select portion of an image. As a further example, when calculating a focus score, the analysis module **656** may calculate the size of a spot derived from the image. The spot can be represented as a Gaussian spot and size can be measured as the full width half maximum (FWHM), in which case smaller spot size is typically correlated with improved focus.

After determining the focused location FL in the test image, the analysis module **656** may then measure or determine the distance XD that the focused location FL is spaced apart or separated from the reference edge **880**. The distance XD may then be correlated to a z-position of the sample area **608** with respect to the sample plane **846**. For example, the analysis module **656** may determine that the distance XD<sub>2</sub> shown in FIG. **58** corresponds to the sample area **608** be located a distance Δz from the sample plane **846**. As such, the sample holder **650** may then be moved the distance Δz to move the sample area **608** within the sample plane **846**. Accordingly, the focused locations FL in test images may be indicative of a position of the sample area **608** with respect to the sample plane **846**. As used herein, the phrase “being indicative of a position of the object (or sample) with respect to the object (or sample) plane” includes using the factor (e.g., the focused location) to provide a more suitable model or algorithm for determining the distance Δz.

FIG. **59** is a block diagram illustrating a method **890** for controlling focus of an optical imaging system. The method **890** includes providing an optical train at **892** having a rotatable mirror that is configured to direct optical signals onto a detector surface. The detector surface may be similar to the detector surface **844**. The optical train may have an object plane, such as the sample plane **846**, that is proximate to an object. The optical train may also have an image plane, such as the image plane **848**, that is proximate to the detector surface. The rotatable mirror may be rotatable between an imaging position and a focusing position.

The method **890** also includes rotating the mirror at **894** to the focusing position and obtaining a test image of the object at **896** when the mirror is in the focusing position. The test image may have an optimal degree-of-focus at a focused location. The focused location may be indicative of a position of the object with respect to the object plane. Furthermore, the method **890** may also include moving the object at **898** toward the object plane based on the focused location.

It is to be understood that the above description is intended to be illustrative, and not restrictive. For example, the above-described embodiments (and/or aspects thereof) may be used in combination with each other. In addition, many modifications may be made to embodiments without departing from the scope of the invention in order to adapt a particular situation or material. While the specific components and processes described herein are intended to define the parameters of the various embodiments, they are by no means limiting and are exemplary embodiments. Many other embodiments will be apparent to those of skill in the art upon reviewing the above description. The scope of the invention should, therefore, be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. In the appended claims, the terms “including” and “in which” are used as the plain-English equivalents of the respective terms “comprising” and “wherein.” Moreover, in the following claims, the terms “first,” “second,” and

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“third,” etc. are used merely as labels, and are not intended to impose numerical requirements on their objects. Further, the limitations of the following claims are not written in means-plus-function format and are not intended to be interpreted based on 35 U.S.C. §112, sixth paragraph, unless and until such claim limitations expressly use the phrase “means for” followed by a statement of function void of further structure.

What is claimed is:

**1.** A fluidic device holder configured to orient a fluidic device with respect to mutually perpendicular X, Y, and Z-axes, the device holder comprising:

a support structure configured to receive a fluidic device, the support structure including a base surface that faces in a direction along the Z-axis and is configured to have the fluidic device positioned thereon;

a plurality of reference surfaces facing in respective directions along an XY-plane;

an alignment assembly comprising an actuator and a movable locator arm that is operatively coupled to the actuator, the locator arm having an engagement end, the actuator moving the locator arm between retracted and biased positions to move the engagement end away from and toward the reference surfaces, wherein the engagement end presses, in a direction along the XY plane, the fluidic device against the reference surfaces when the locator arm is in the biased position such that the fluidic device is held between the engagement end and the reference surfaces in a fixed position with respect to the support structure, wherein the engagement end rotates within the XY-plane when the locator arm is moved between the retracted and biased positions.

**2.** The fluidic device holder of claim **1**, wherein the reference surfaces include first and second reference surfaces that face in perpendicular directions with respect to each other, the first reference surface stopping movement of the fluidic device in a direction along the X axis when the locator arm is in the biased position, the second reference surface stopping movement of the fluidic device in a direction along the Y axis when the locator arm is in the biased position, the engagement end providing a force when in the biased position that has a component along the X axis and a component along the Y axis.

**3.** The fluidic device holder of claim **1**, further comprising a removable cover assembly that is operatively coupled to the actuator such that movement of the cover assembly causes the actuator to move the locator arm to the biased position as the cover assembly is mounted over the fluidic device, the actuator moving the locator arm to the retracted position as the cover assembly is demounted from the fluidic device.

**4.** The fluidic device holder of claim **3**, wherein the cover assembly is movable back and forth between an open position and a closed position along the Z-axis, the cover assembly engaging and holding the fluidic device against the base surface when in the closed position, the locator arm being in the biased position when the cover assembly is in the closed position.

**5.** The fluidic device holder of claim **4**, wherein the locator arm is at the biased position before the cover assembly is at the closed position.

**6.** The fluidic device holder of claim **1**, further comprising a removable cover assembly that includes a flow system having fluidic ports, the cover assembly being movable between an open position and a closed position, the cover assembly engaging and pressing the fluidic device toward the base surface when mounted over the fluidic device in the closed position, wherein the fluidic ports couple to corresponding inlet and outlet ports of the fluidic device when the cover



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assembly is mounted over the fluidic device in the closed position, the locator arm being in the biased position when the cover assembly is in the closed position.

7. A fluidic system that includes the fluidic device holder of claim 6, wherein the flow system further comprises a removable fluid storage device, the fluid storage device comprising a tray, and a plurality of component wells in the tray, individual wells of the plurality having an opening at the top of the well.

8. The fluidic system of claim 7, wherein the removable fluid storage device is in a fluid storage system, the fluid storage system comprising:

- (a) an enclosure having a cavity;
  - (b) a temperature control assembly configured to regulate a temperature within the cavity; and
  - (c) a fluid removal assembly comprising a gripper for the removable fluid storage device, an elevator mechanism, and an array of sipper tubes,
- wherein the elevator mechanism comprises a drive motor configured to move the array of sipper tubes bi-directionally relative to the gripper.

9. The fluidic system of claim 8, wherein the plurality of component wells comprises a well containing a polymerase, a well containing primer nucleic acids, a well containing nucleic acid denaturants, wells containing nucleotides having reversible terminator moieties and fluorescent labels and a well comprising a nucleic acid sample to be sequenced.

10. The fluidic system of claim 8, wherein the opening at the top of the individual component well comprises a rim that projects a distance away from the upper surface of the tray.

11. The fluidic system of claim 8, wherein the fluid storage device further comprises a protective foil covering the opening at the top of individual wells and the sipper tubes are configured to pierce the protective foil.

12. The fluidic device holder of claim 1, wherein the support structure comprises a thermal module having the base surface, the thermal module configured to transfer thermal energy through the base surface.

13. A fluidic system that includes the fluidic device holder of claim 1, further comprising the fluidic device, the fluidic device having a housing and a flow cell that is floatably held by the housing, wherein the locator arm directly engages the housing of the fluidic device and the reference surfaces directly engage the flow cell.

14. A fluidic system that includes the fluidic device holder of claim 1, further comprising the fluidic device, the fluidic device comprising:

- a flow cell including inlet and outlet ports and a flow channel extending therebetween, the flow cell configured to hold a sample-of-interest;
- a housing having a reception space that is configured to receive the flow cell, the reception space being sized and shaped to permit the flow cell to float relative to the housing when the locator arm is in the retracted position; and
- a gasket coupled to the housing, the gasket having inlet and outlet passages and comprising a compressible material, the gasket being positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively when the locator arm is in the biased position.

15. The fluidic system of claim 14, wherein the fluidic device further comprises a cover member that is rotatably coupled to the housing and includes the gasket, the gasket being rotatable about an axis of rotation between a mounted position and a disengaged position, the inlet and outlet pas-

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sages being approximately aligned with the inlet and outlet ports when the gasket is in the mounted position and when the locator arm is in the biased position.

16. The fluidic system of claim 14, wherein the flow cell has a plurality of edges that extend along a cell plane and define a perimeter of the flow cell, the gasket configured to press against one of the edges when the inlet and outlet passages are approximately aligned and when the locator arm is in the biased position, the gasket limiting movement of the flow cell within the reception space along the cell plane.

17. The fluidic system of claim 16, wherein the flow cell has first and second cell sides that face in opposite directions, the first and second cell sides extending along the cell plane, wherein the gasket also presses against one of the first and second cell sides thereby limiting movement in a direction that is perpendicular to the cell plane.

18. The fluidic system of claim 16, wherein the flow channel comprises an imaging portion and a non-imaging portion, wherein imaging and non-imaging portions are fluidically joined by a curved portion, the imaging and non-imaging portions extending parallel to each other,

wherein the inlet port is in fluid communication with the imaging portion of the flow channel, and wherein the outlet port is in fluid communication with the non-imaging portion of the flow channel.

19. The fluidic system of claim 18, wherein the width of the imaging portion of the flow channel is larger than the width of the non-imaging portion of the flow channel.

20. The fluidic system of claim 18, wherein a plurality of DNA clusters is on the surface of the imaging portion of the flow channel.

21. The fluidic system of claim 18, wherein the curved portion of the flow channel comprises a tapering portion, an intermediate portion, and a downstream portion,

wherein the tapering portion connects the imaging portion with the intermediate portion, and wherein the width of the tapering portion reduces in size from the imaging portion of the flow channel to the intermediate portion.

22. The fluidic system of claim 18, wherein the surface of the flow channel comprises a transparent material.

23. A method comprising:

providing the fluidic device holder of claim 1;  
positioning a fluidic device on the base surface of the fluidic device holder between the plurality of reference surfaces, the device having a sample area; and  
moving the locator arm to the biased position, the locator arm pressing the device against the reference surfaces such that the device is held in a fixed position.

24. The fluidic device holder of claim 1, wherein the reference surfaces have fixed positions with respect to each other and the base surface when the locator arm is in the biased position or in the retracted position.

25. The fluidic device holder of claim 1, wherein the locator arm provides a resilient biasing force having a stored potential energy that actively presses the engagement end against the fluidic device when the locator arm is in the biased position.

26. The fluidic device holder of claim 3, wherein the locator arm rotates about an axis between the retracted and biased positions and wherein the first and second reference surfaces have fixed positions relative to each other and the base surface when the locator arm is in the biased position or in the retracted position.

27. A fluidic system that includes the fluidic device holder of claim 1, further comprising the fluidic device, wherein the fluidic device holder has a loading space that is defined by the



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base surface and is configured to receive the fluidic device, the loading space being greater than the fluidic device along the X-axis and greater than the fluidic device along the Y-axis, the locator arm moving the fluidic device, when the fluidic device is mispositioned within the loading space, to the fixed position.

**28.** The fluidic device holder of claim **3**, wherein the cover assembly is rotatable back and forth between an open position and a closed position, the cover assembly moving toward the base surface along the Z-axis when moved to the closed position and moving away from the base surface along the Z-axis when moved to the open position.

**29.** The fluidic device holder of claim **6**, wherein the cover assembly includes a cover housing that defines a viewing space, the viewing space existing above the fluidic device and being sized and shaped to permit an imaging lens to be positioned above the fluidic device when the cover assembly is in the closed position.

**30.** A fluidic device holder configured to orient a fluidic device with respect to mutually perpendicular X, Y, and Z-axes, the device holder comprising:

a support structure configured to receive a fluidic device, the support structure including a base surface that faces in a direction along the Z-axis and is configured to have the fluidic device positioned thereon;

a plurality of reference surfaces facing in respective directions along an XY-plane;

an alignment assembly comprising an actuator and a movable locator arm that is operatively coupled to the actuator, the locator arm having an engagement end, the actuator moving the locator arm between retracted and biased positions to move the engagement end away from and toward the reference surfaces, wherein the engagement end presses, in a direction along the XY plane, the fluidic device against the reference surfaces when the locator arm is in the biased position such that the fluidic device is held between the engagement end and the reference surfaces in a fixed position with respect to the support structure, wherein the locator arm includes a finger, the finger including the engagement end.

**31.** The fluidic device holder of claim **30**, wherein the engagement end moves within the XY-plane when the locator arm is moved between the retracted and biased positions.

**32.** The fluidic device holder of claim **30**, wherein the reference surfaces include first and second reference surfaces that face in perpendicular directions with respect to each other, the first reference surface stopping movement of the fluidic device in a direction along the X axis when the locator arm is in the biased position, the second reference surface stopping movement of the fluidic device in a direction along the Y axis when the locator arm is in the biased position, the engagement end providing a force when in the biased position that has a component along the X axis and a component along the Y axis.

**33.** The fluidic device holder of claim **32**, wherein the first and second reference surfaces have fixed positions relative to each other and the base surface when the locator arm is in the biased position or in the retracted position.

**34.** The fluidic device holder of claim **30**, further comprising a removable cover assembly that is operatively coupled to the actuator such that movement of the cover assembly causes the actuator to move the locator arm to the biased position as the cover assembly is mounted over the fluidic device, the actuator moving the locator arm to the retracted position as the cover assembly is demounted from the fluidic device.

**35.** The fluidic device holder of claim **34**, wherein the cover assembly is movable back and forth between an open position

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and a closed position along the Z-axis, the cover assembly engaging and holding the fluidic device against the base surface when in the closed position, the locator arm being in the biased position when the cover assembly is in the closed position.

**36.** The fluidic device holder of claim **34**, wherein the cover assembly is rotatable back and forth between an open position and a closed position, the cover assembly moving toward the base surface along the Z-axis when moved to the closed position and moving away from the base surface along the Z-axis when moved to the open position.

**37.** The fluidic device holder of claim **30**, further comprising a removable cover assembly that includes a flow system having fluidic ports, the cover assembly being movable between an open position and a closed position, the cover assembly engaging and pressing the fluidic device toward the base surface when mounted over the fluidic device in the closed position, wherein the fluidic ports couple to corresponding inlet and outlet ports of the fluidic device when the cover assembly is mounted over the fluidic device in the closed position, the locator arm being in the biased position when the cover assembly is in the closed position.

**38.** A fluidic system that includes the fluidic device holder of claim **30**, further comprising the fluidic device, the fluidic device having a housing and a flow cell that is floatably held by the housing, wherein the locator arm directly engages the housing of the fluidic device and the reference surfaces directly engage the flow cell.

**39.** A fluidic system that includes the fluidic device holder of claim **30**, further comprising the fluidic device, the fluidic device comprising:

a flow cell including inlet and outlet ports and a flow channel extending therebetween, the flow cell configured to hold a sample-of-interest;

a housing having a reception space that is configured to receive the flow cell, the reception space being sized and shaped to permit the flow cell to float relative to the housing when the locator arm is in the retracted position; and

a gasket coupled to the housing, the gasket having inlet and outlet passages and comprising a compressible material, the gasket being positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively when the locator arm is in the biased position.

**40.** The fluidic system of claim **39**, wherein the fluidic device further comprises a cover member that is rotatably coupled to the housing and includes the gasket, the gasket being rotatable about an axis of rotation between a mounted position and a disengaged position, the inlet and outlet passages being approximately aligned with the inlet and outlet ports when the gasket is in the mounted position and when the locator arm is in the biased position.

**41.** The fluidic system of claim **39**, wherein the flow cell has a plurality of edges that extend along a cell plane and define a perimeter of the flow cell, the gasket configured to press against one of the edges when the inlet and outlet passages are approximately aligned and when the locator arm is in the biased position, the gasket limiting movement of the flow cell within the reception space along the cell plane.

**42.** The fluidic device holder of claim **30**, wherein the locator arm provides a resilient biasing force having a stored potential energy that actively presses the engagement end against the fluidic device when the locator arm is in the biased position.



43. A fluidic system that includes the fluidic device holder of claim 30, further comprising the fluidic device, wherein the fluidic device holder has a loading space that is defined by the base surface and is configured to receive the fluidic device, the loading space being greater than the fluidic device along the X-axis and greater than the fluidic device along the Y-axis, the locator arm moving the fluidic device, when the fluidic device is mispositioned within the loading space, to the fixed position.

44. A method comprising:  
providing the fluidic device holder of claim 30;  
positioning a fluidic device on the base surface of the fluidic device holder between the plurality of reference surfaces, the device having a sample area; and  
moving the locator arm to the biased position, the locator arm pressing the device against the reference surfaces such that the device is held in a fixed position.

\* \* \* \* \*



UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 8,951,781 B2  
APPLICATION NO. : 13/273666  
DATED : February 10, 2015  
INVENTOR(S) : Erik Williamson et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title page item (12), should read, Williamson, et al.

Title page item (75), Inventors, Line 1, delete “Mark T. Reed, Menlo Park, CA (US);”

Title page item (75), Inventors, Line 2, delete “Eric” and insert -- Erik --

Title page item (75), Inventors, Lines 5-13, delete

“Dale Buermann, San Diego, CA (US);

Alexander P. Kindwall, Pleasanton, CA (US);

Frederick Erie, Encinitas, CA (US);

Mark Pratt, San Mateo, CA (US);

Jason Harris, Hayward, CA (US);

Andrew James Carson, Carlsbad, CA (US);

Stanley S. Hong, Hayward, CA (US);

Jason Bryant, Essex (GB);

Mark Wang, San Diego, CA (US);”

Title page item (75), Inventors, Line 14, after inventor Drew Verkade, Carlsbad, CA (US),  
insert -- Mark Reed, Menlo Park, CA (US); --, therefor

In the Claims

Column 54, Line 59, in Claim 26, delete “claim 3,” and insert -- claim 2, --, therefor

Signed and Sealed this  
Sixteenth Day of June, 2015

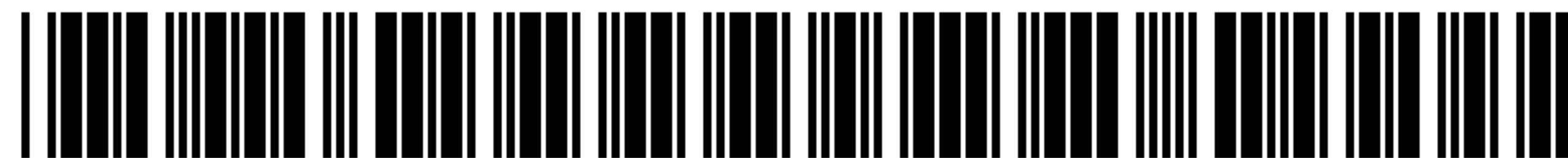


Michelle K. Lee  
*Director of the United States Patent and Trademark Office*



# **EXHIBIT 4**





US011117130B2

(12) **United States Patent**  
**Williamson et al.**

(10) **Patent No.: US 11,117,130 B2**  
(45) **Date of Patent: Sep. 14, 2021**

(54) **SYSTEMS, METHODS, AND APPARATUSES TO IMAGE A SAMPLE FOR BIOLOGICAL OR CHEMICAL ANALYSIS**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **16/874,412**

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(65) **Prior Publication Data**

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#### Related U.S. Application Data

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**B01L 3/00** (2006.01)  
**B01L 9/00** (2006.01)  
(Continued)

(52) **U.S. Cl.**  
CPC ..... **B01L 3/502715** (2013.01); **B01L 9/527** (2013.01); **G01N 21/05** (2013.01);  
(Continued)

(58) **Field of Classification Search**  
CPC ..... B01L 3/50273; B01L 3/502715  
See application file for complete search history.

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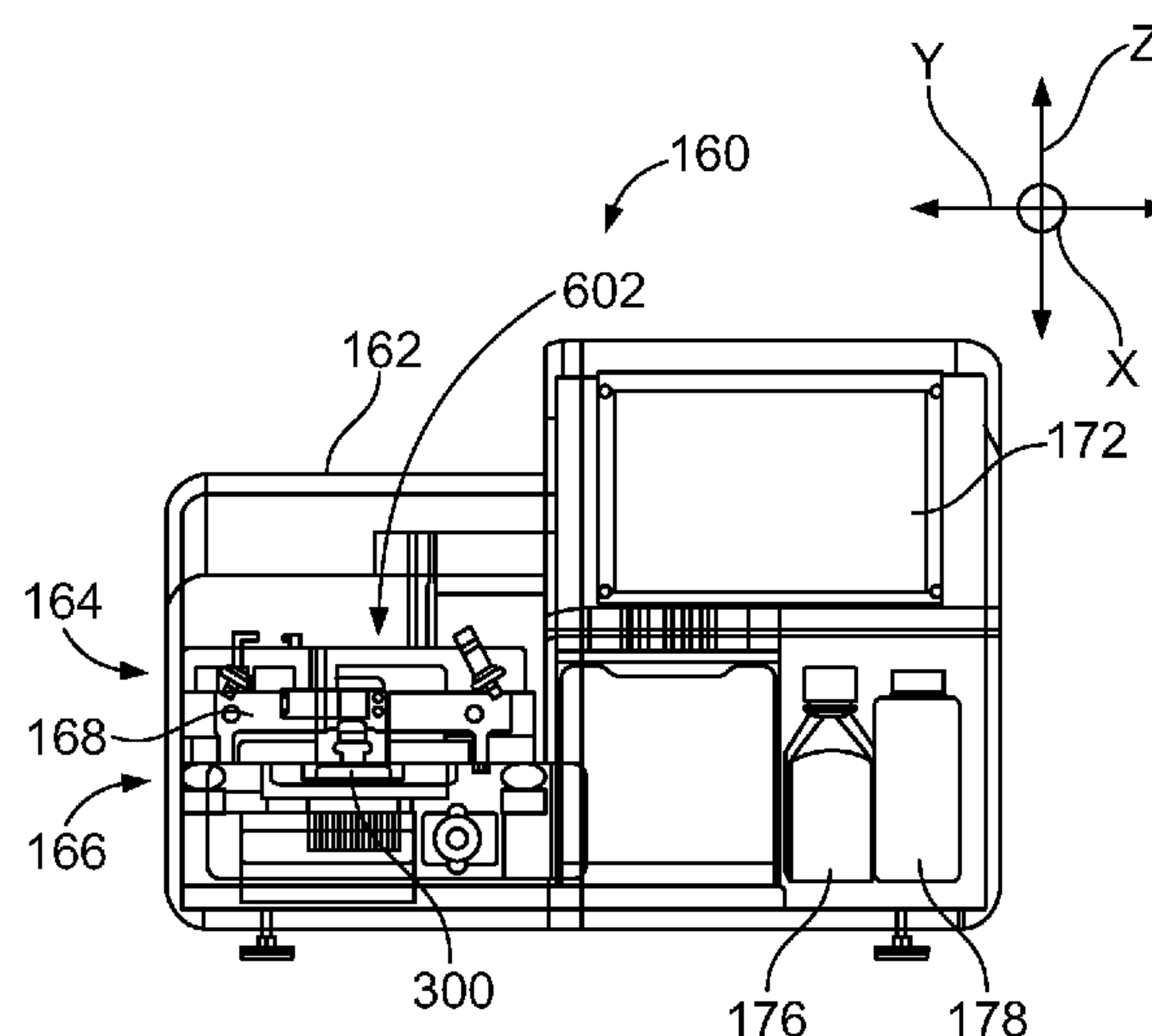
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(57) **ABSTRACT**

A fluidic device holder configured to orient a fluidic device. The device holder includes a support structure configured to receive a fluidic device. The support structure includes a base surface that faces in a direction along the Z-axis and is configured to have the fluidic device positioned thereon. The device holder also includes a plurality of reference surfaces facing in respective directions along an XY-plane. The device holder also includes an alignment assembly having an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end away from and toward the reference surfaces. The locator arm is configured to hold the fluidic device against the reference surfaces when the locator arm is in the biased position.

**26 Claims, 39 Drawing Sheets**





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(51)	<b>Int. Cl.</b>				
	<b>G01N 21/05</b> (2006.01)				
	<b>B01L 7/00</b> (2006.01)				
(52)	<b>U.S. Cl.</b>				
	CPC ..... <b>B01L 3/50273</b> (2013.01); <b>B01L 7/52</b> (2013.01); <b>B01L 2200/025</b> (2013.01); <b>B01L 2200/027</b> (2013.01); <b>B01L 2200/04</b> (2013.01); <b>B01L 2200/0689</b> (2013.01); <b>B01L 2300/022</b> (2013.01); <b>B01L 2300/041</b> (2013.01); <b>B01L 2300/043</b> (2013.01); <b>B01L 2300/0816</b> (2013.01); <b>B01L 2300/0877</b> (2013.01); <b>G01N 2021/058</b> (2013.01); <b>Y10T 436/25</b> (2015.01)				
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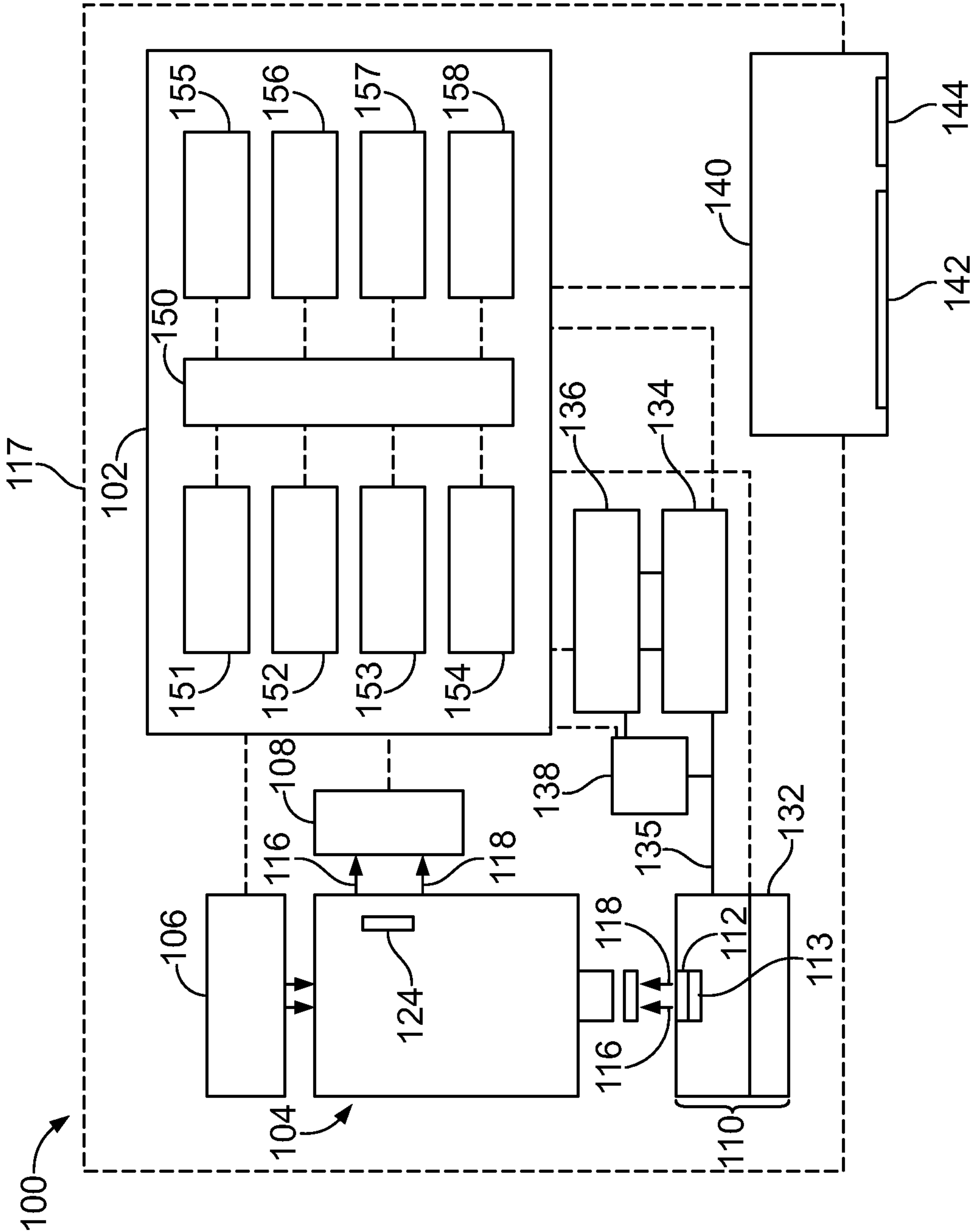


FIG. 1



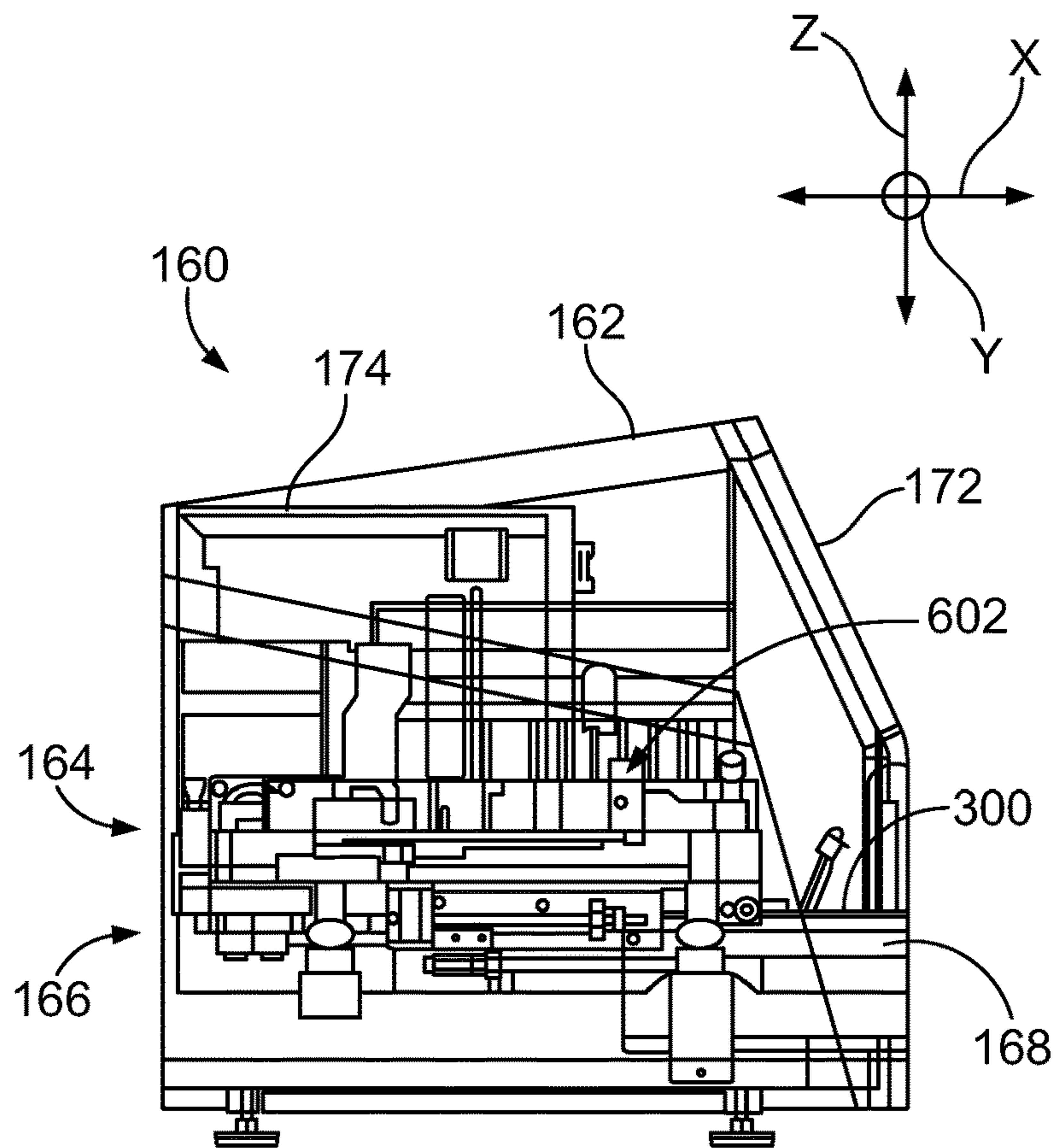


FIG. 2

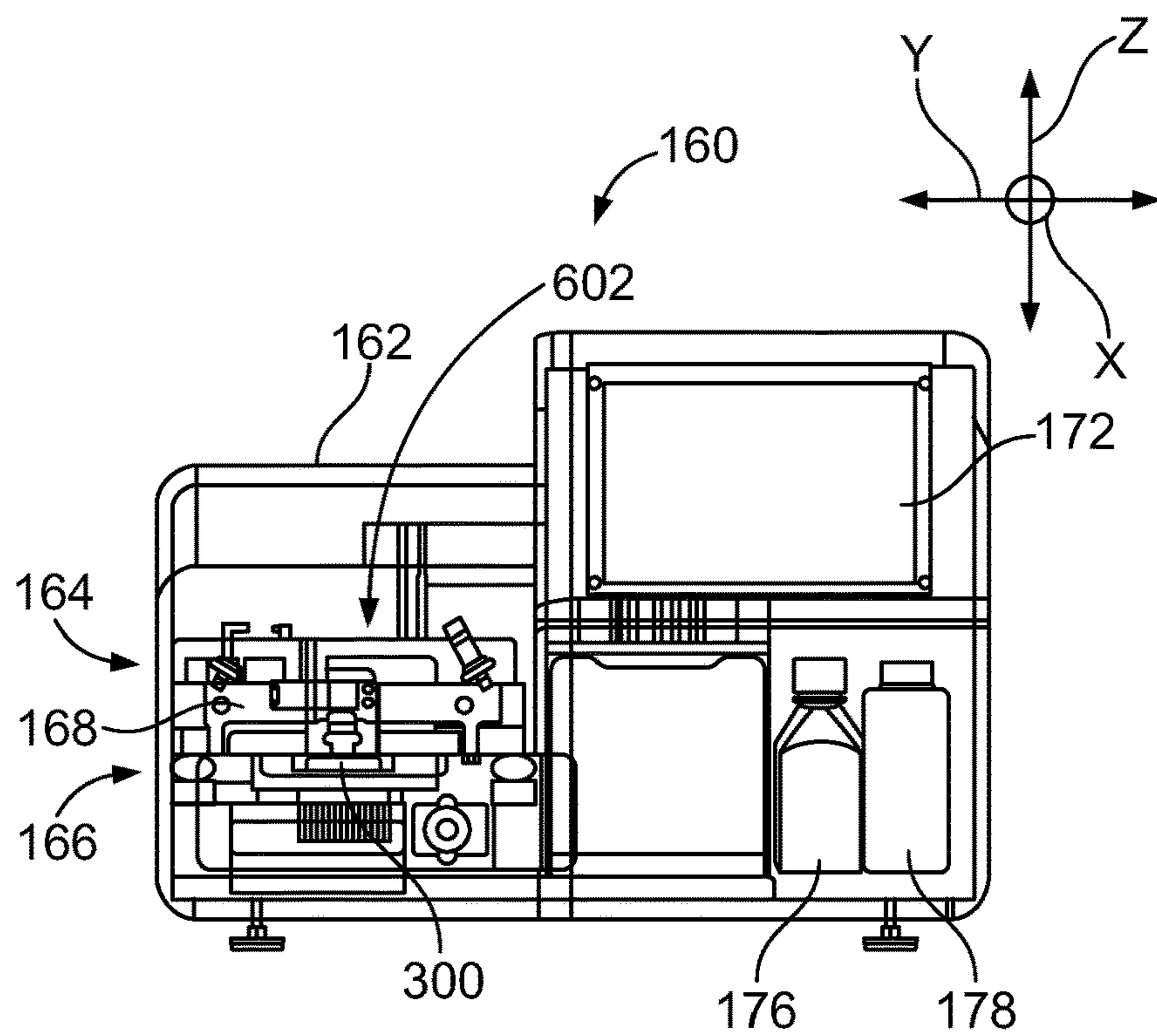


FIG. 3



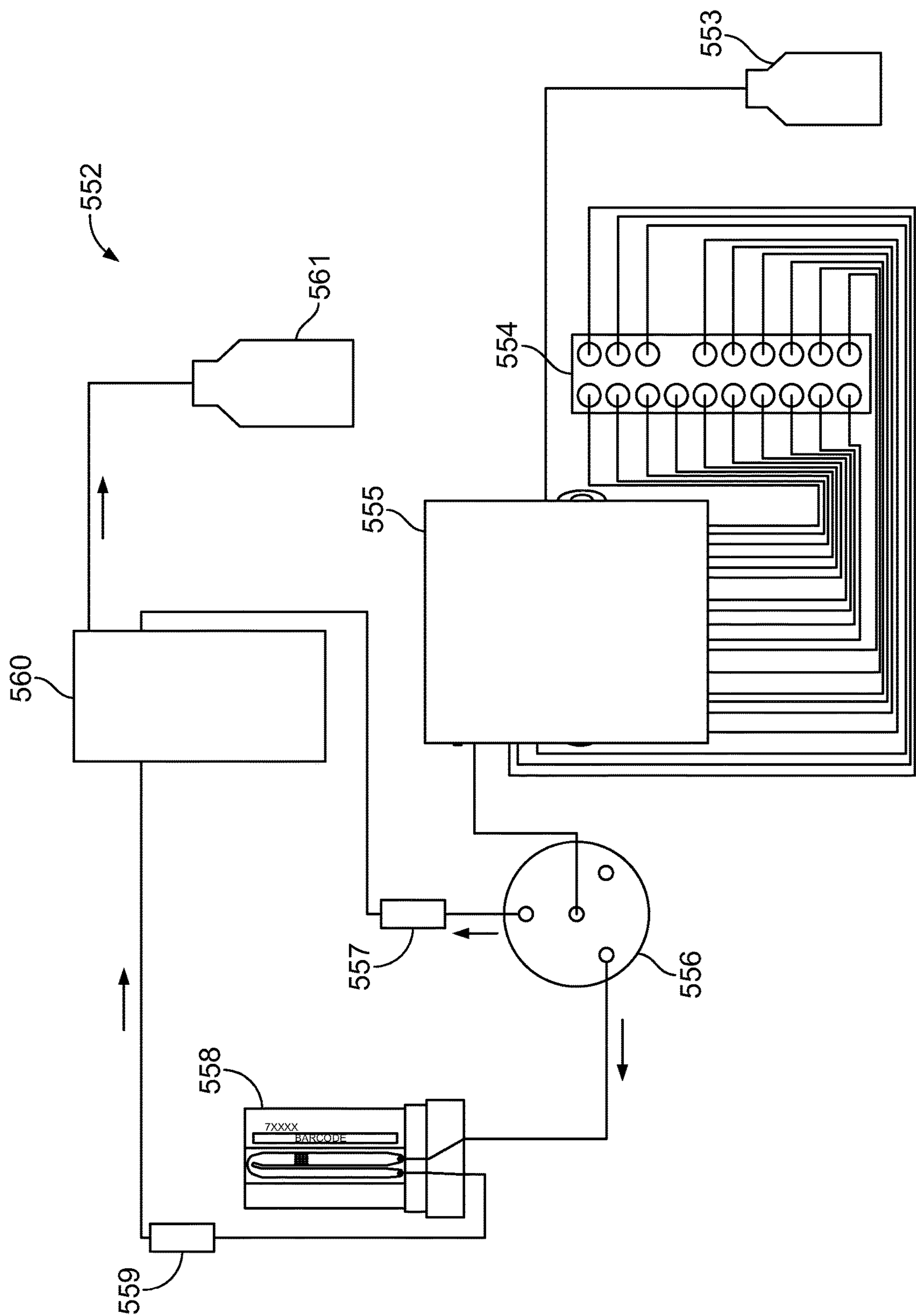


FIG. 4



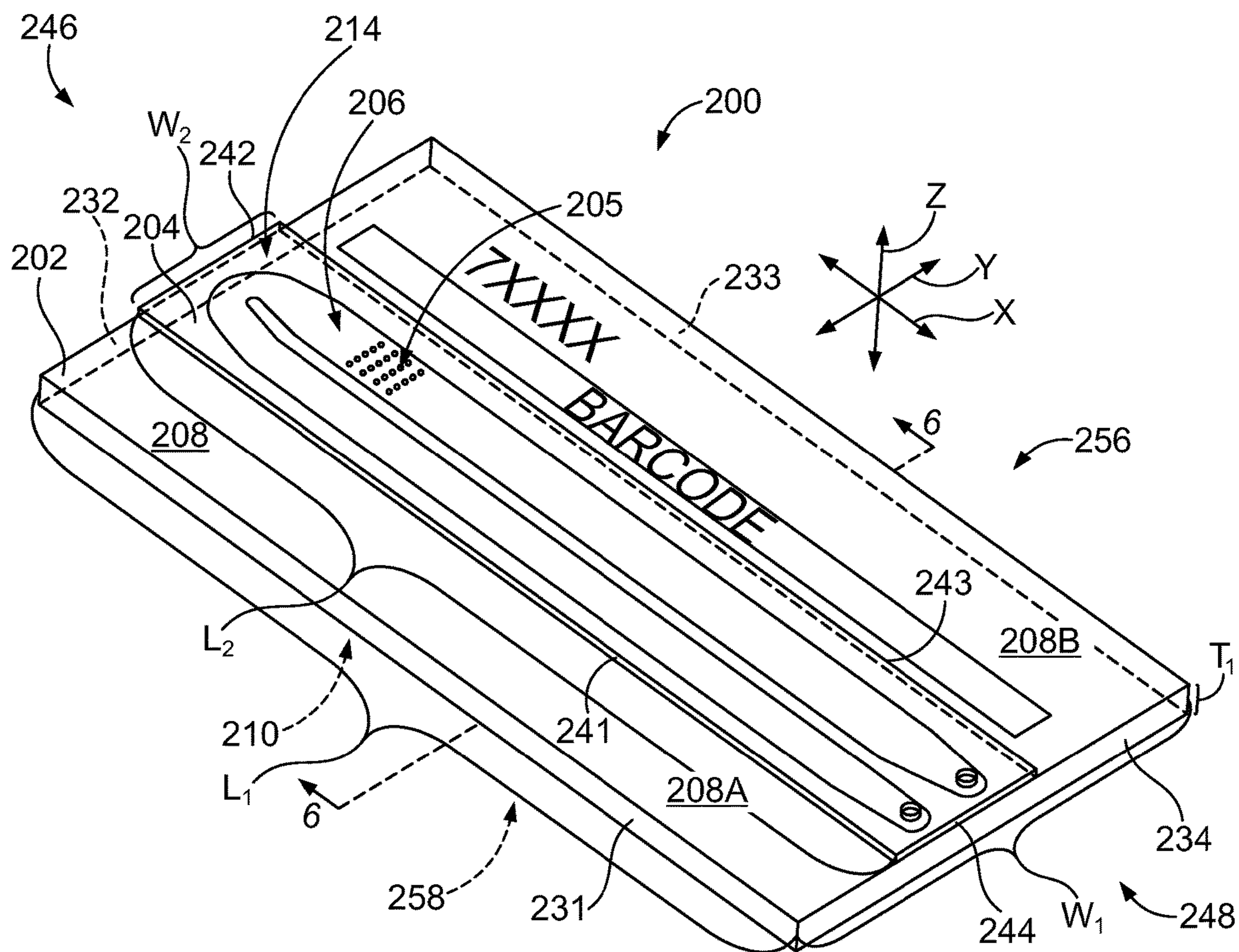
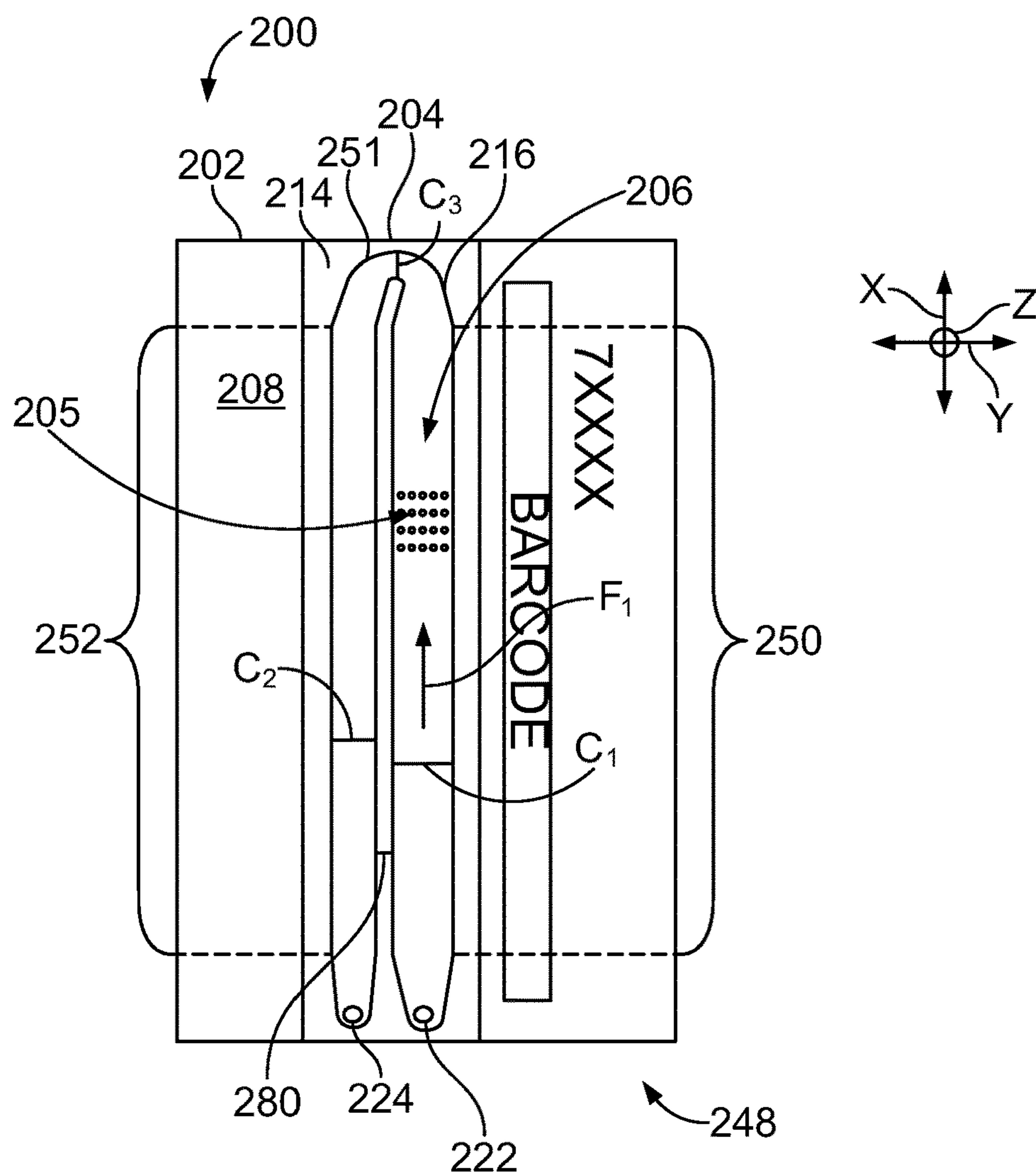
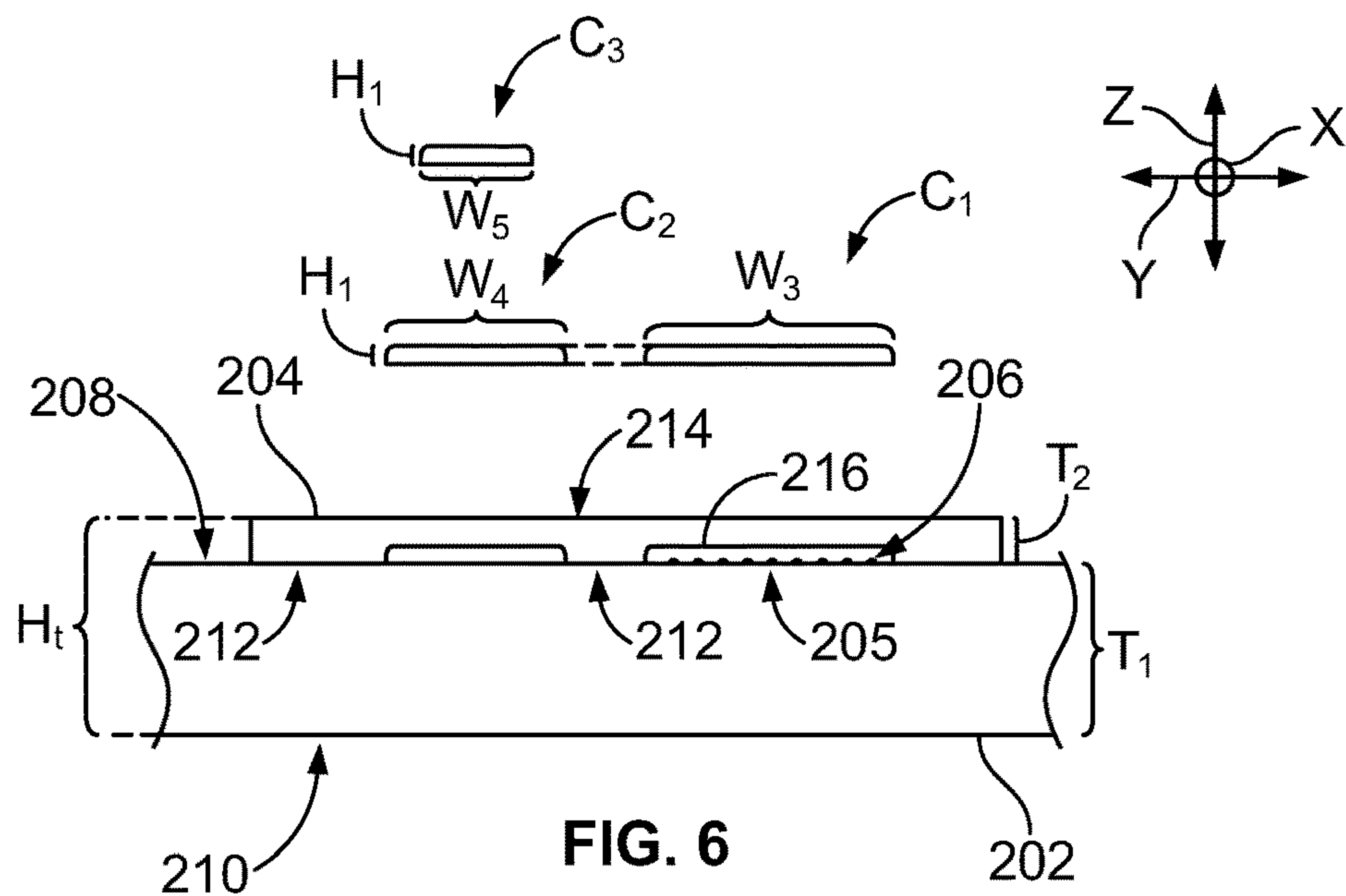


FIG. 5







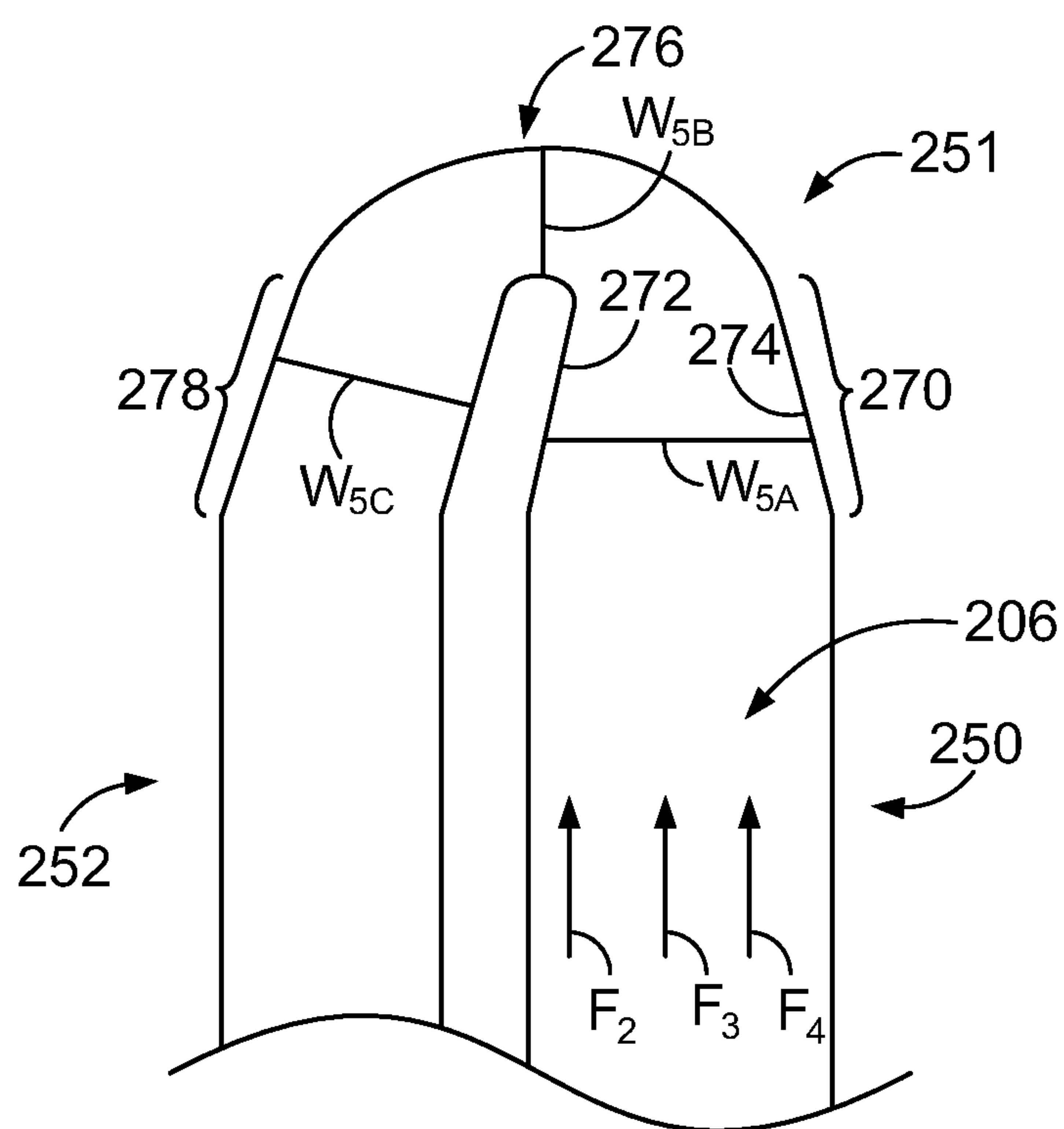


FIG. 8



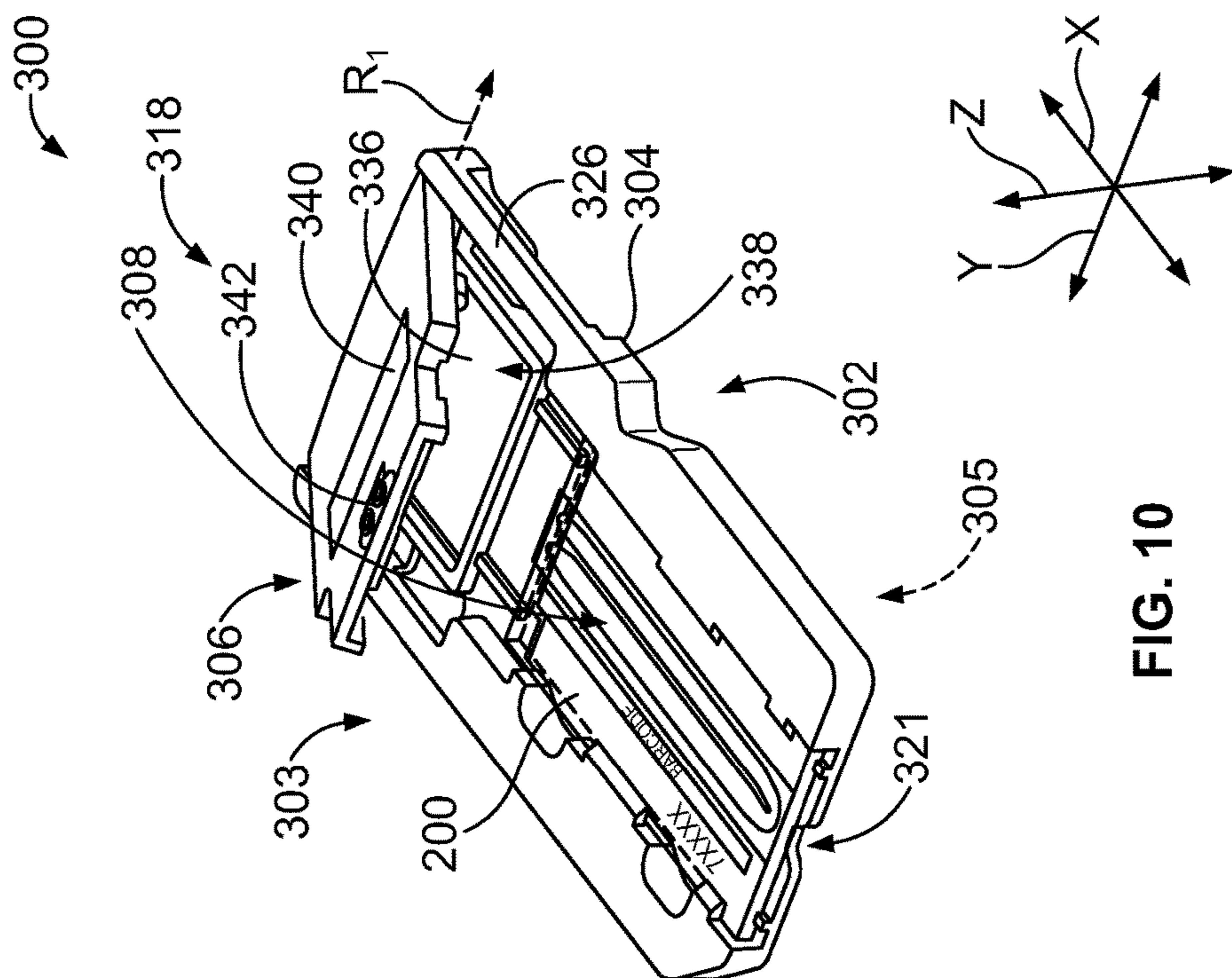


FIG. 10

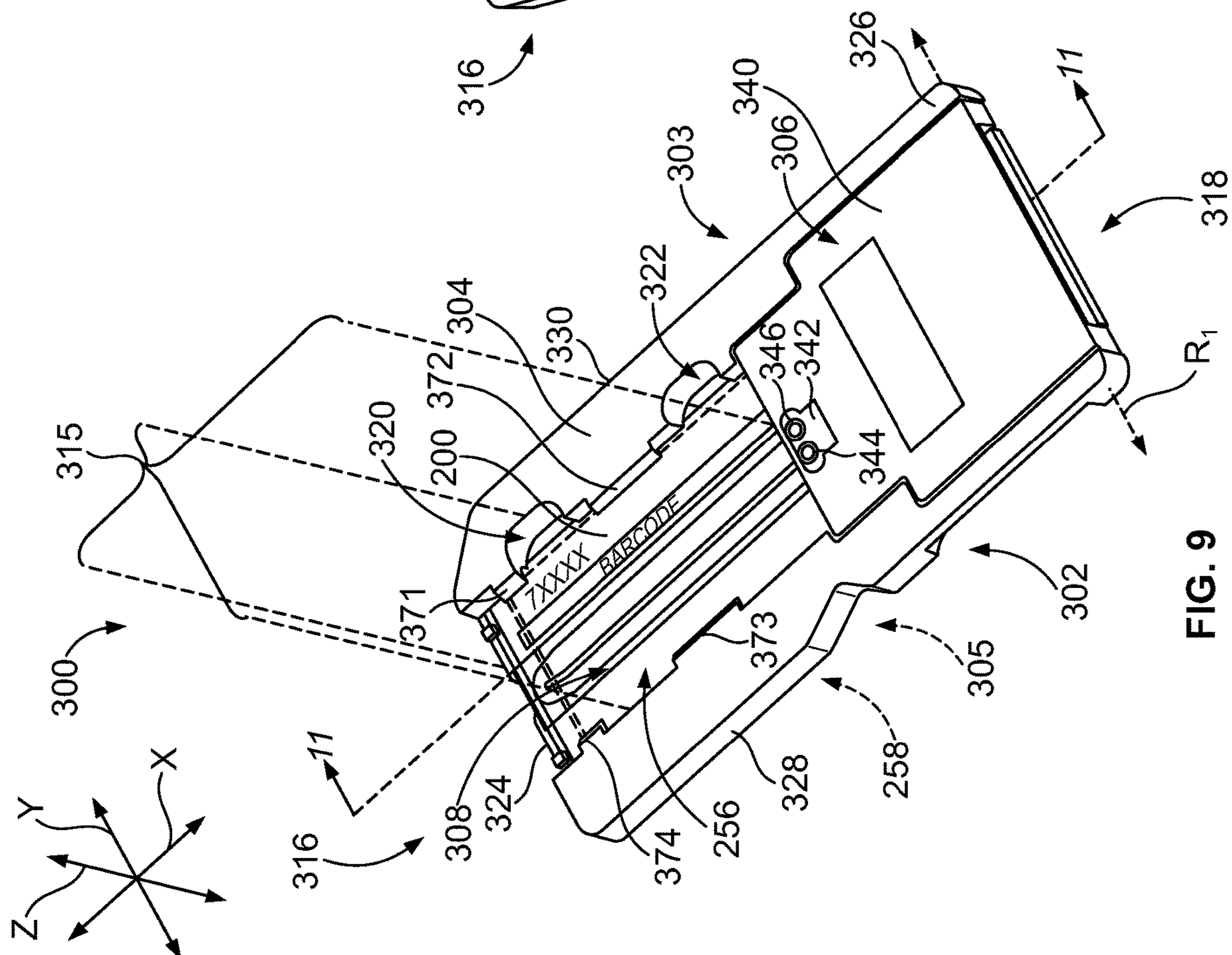


FIG. 9







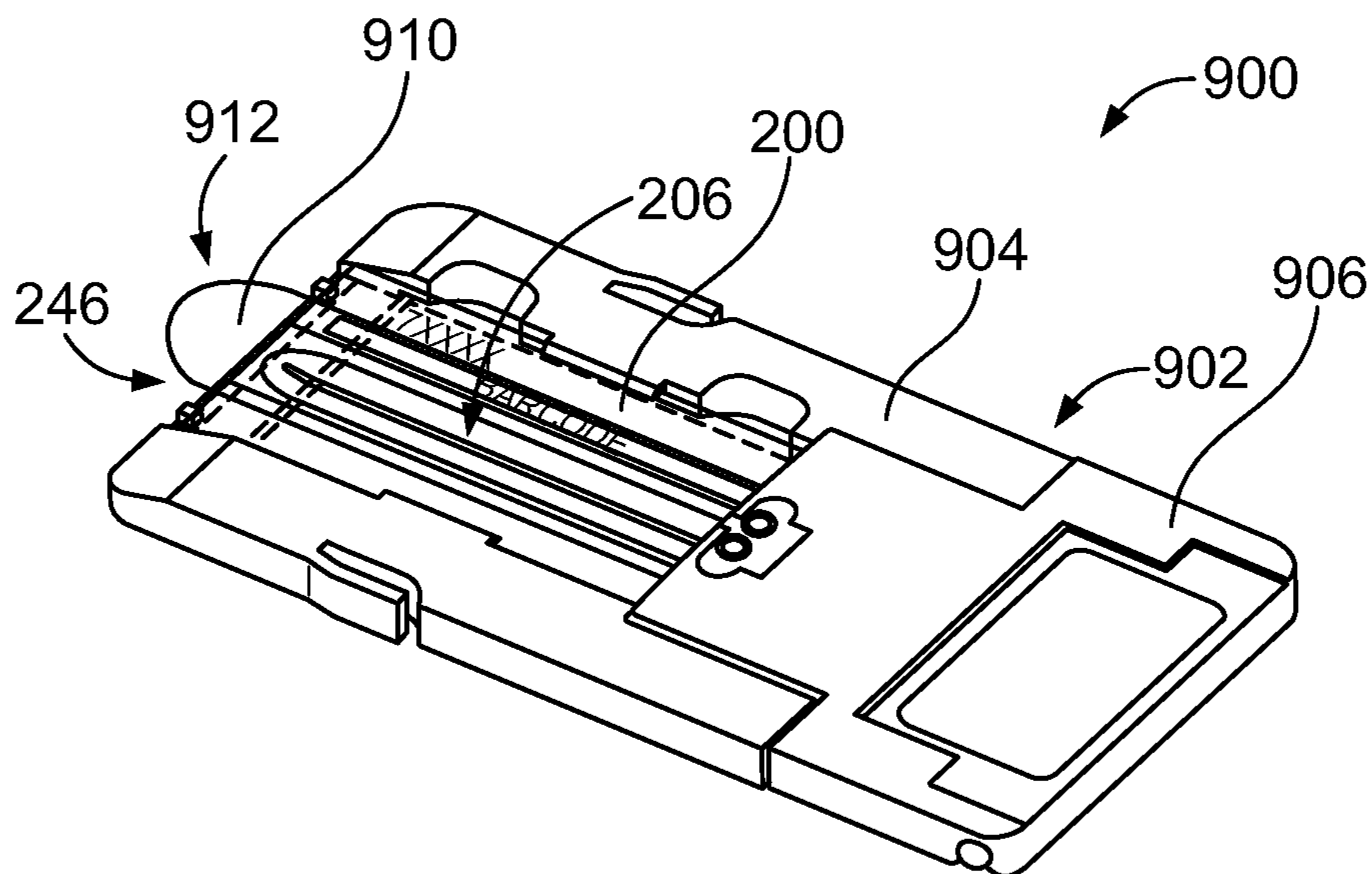


FIG. 12

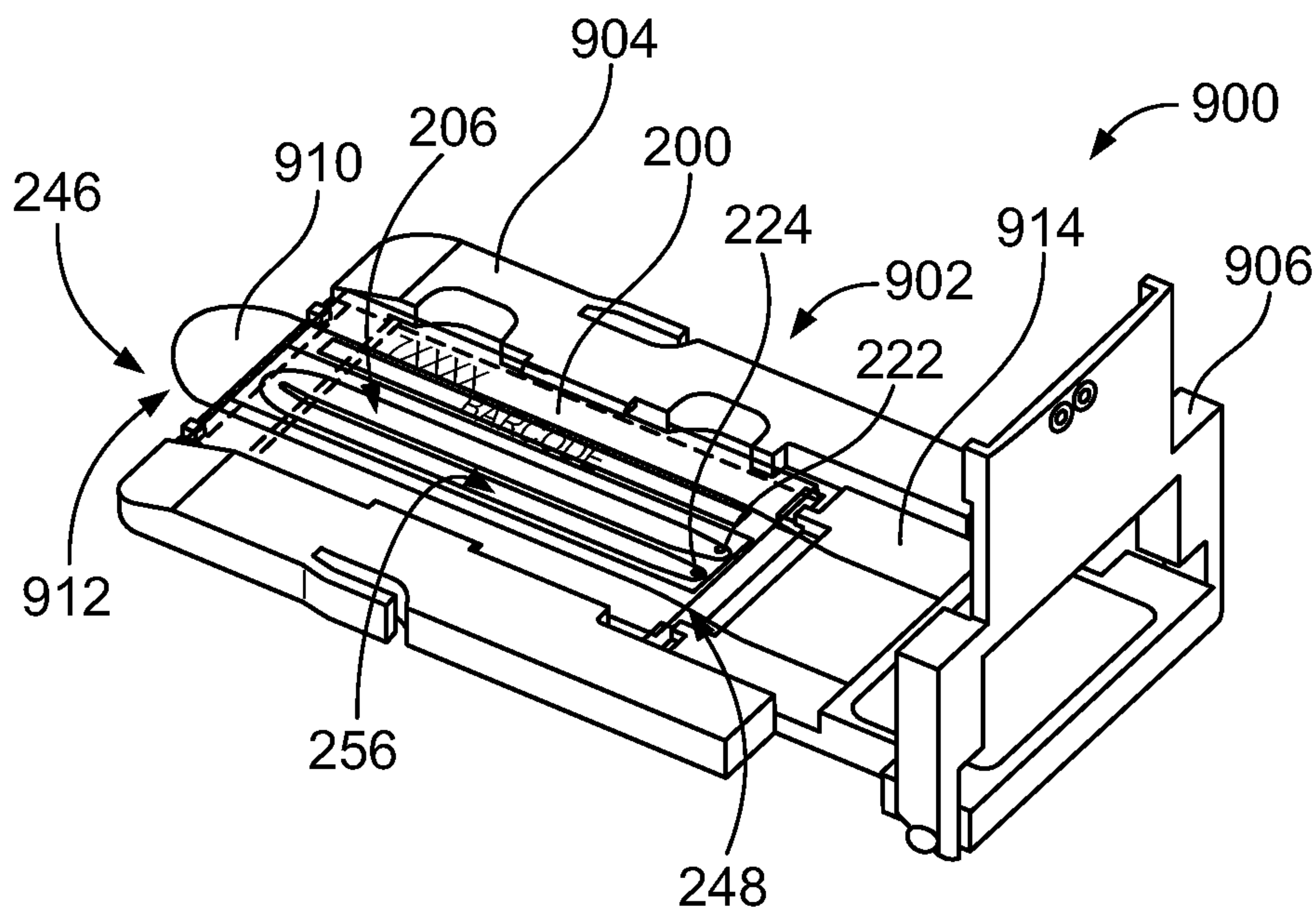


FIG. 13



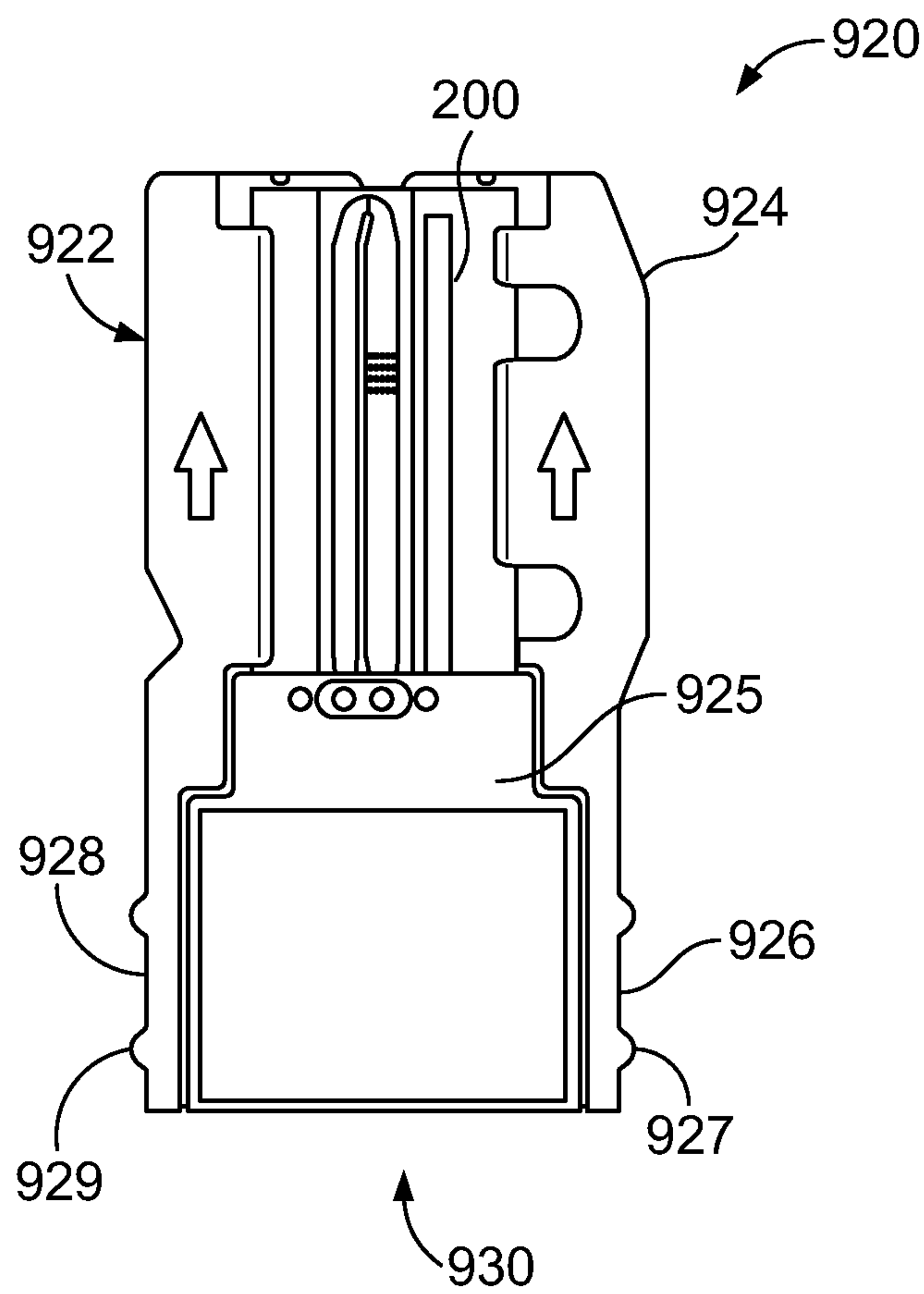


FIG. 14

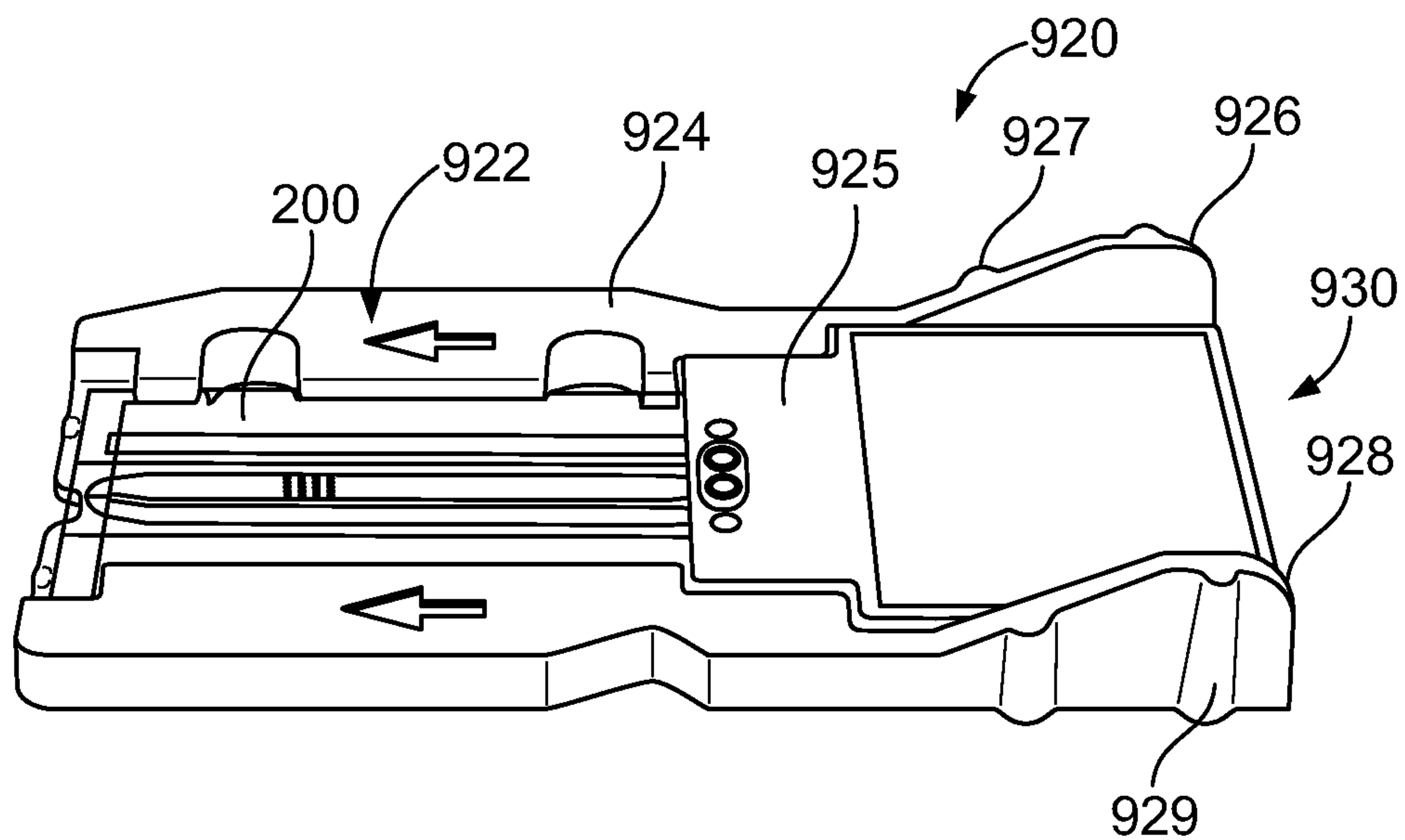


FIG. 15



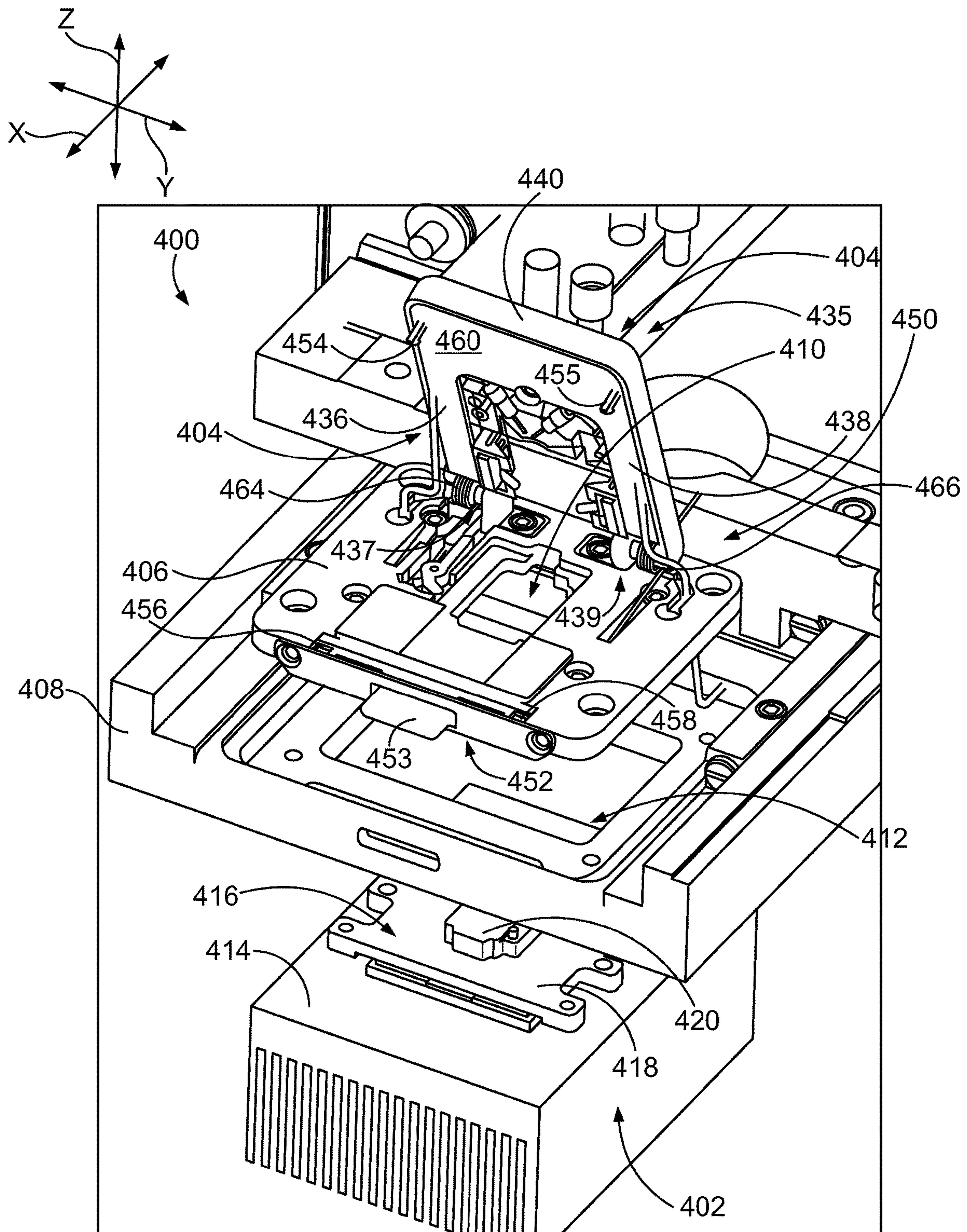
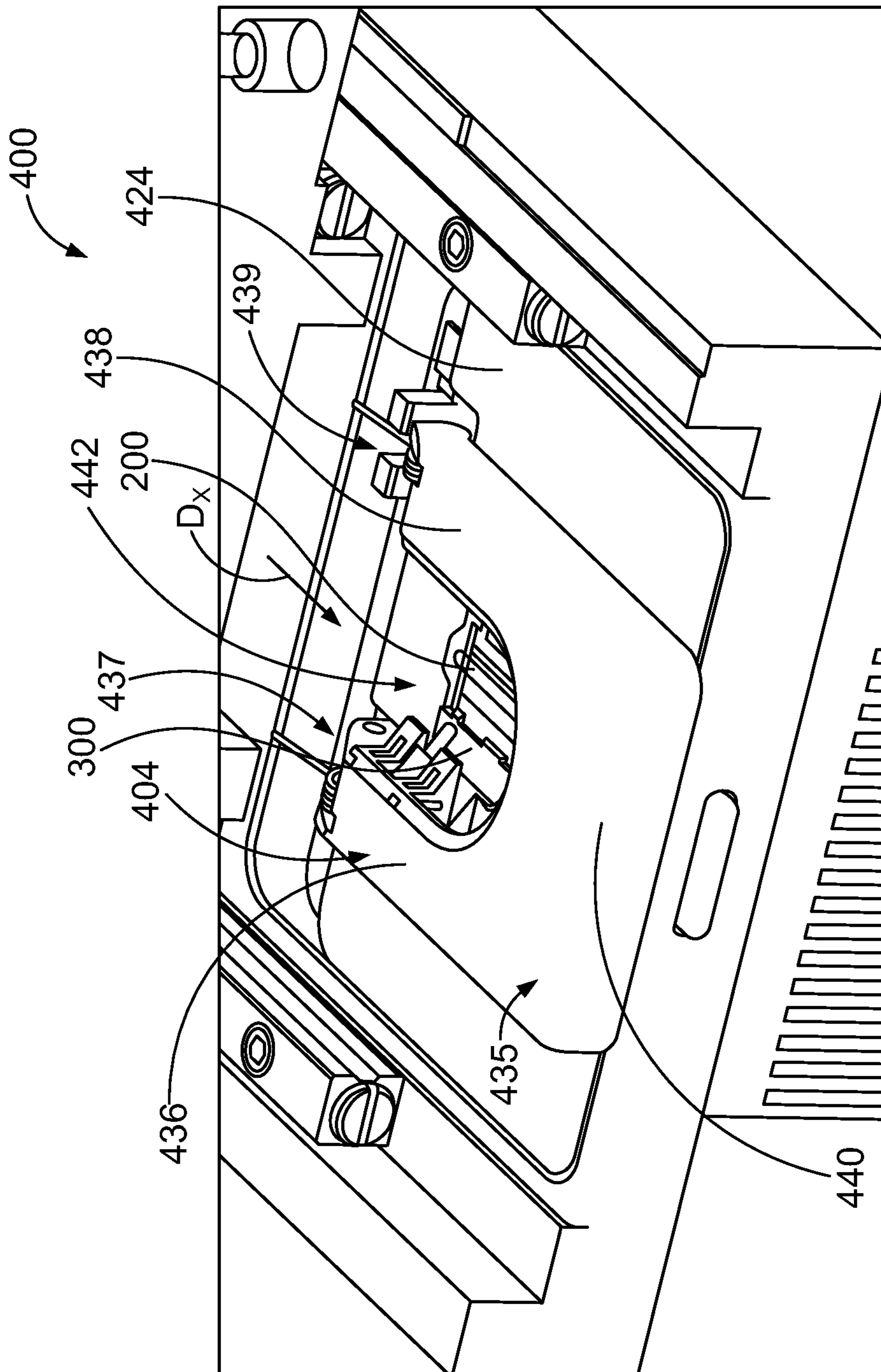


FIG. 16





**FIG. 17**



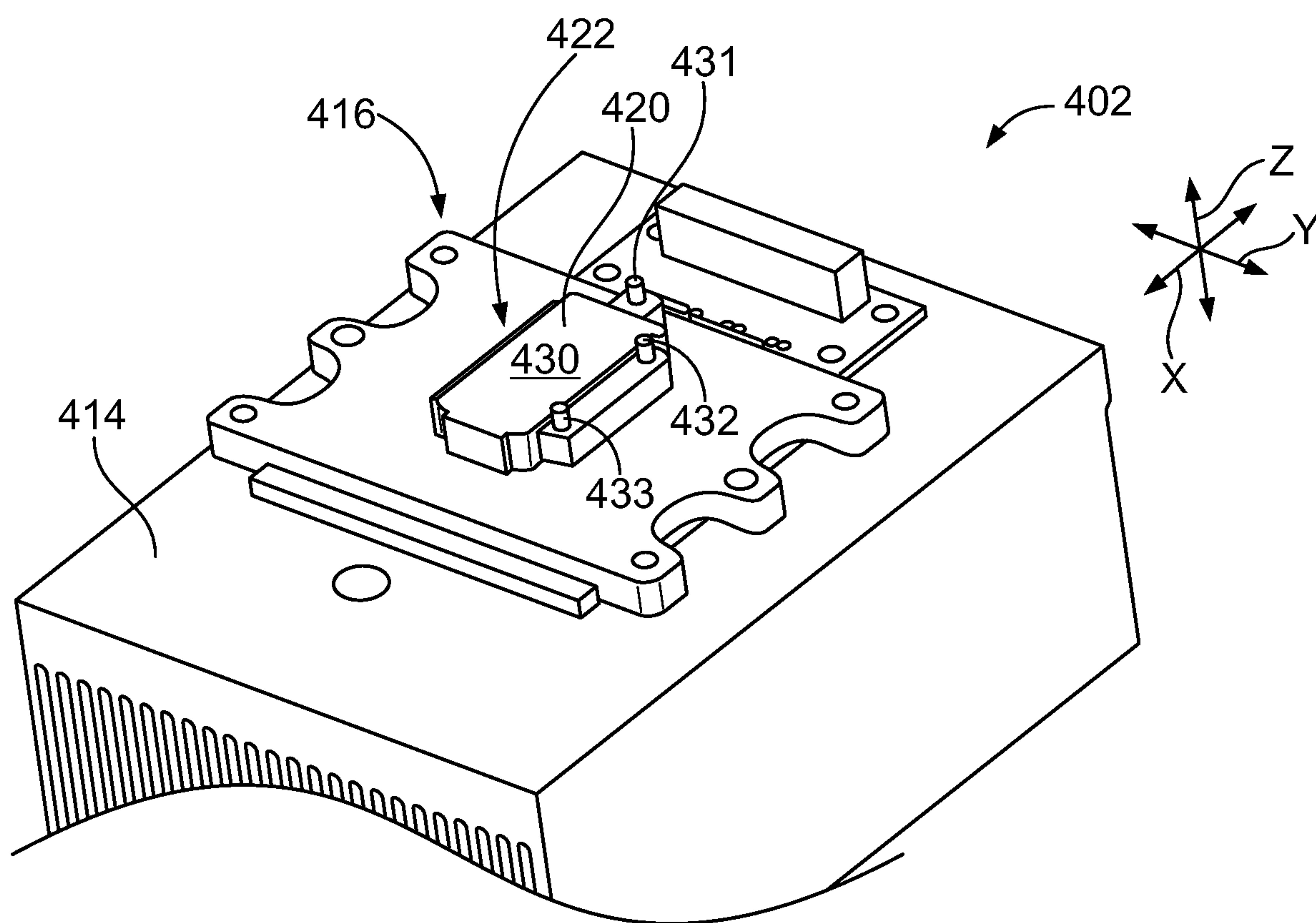


FIG. 18



FIG. 19



FIG. 20



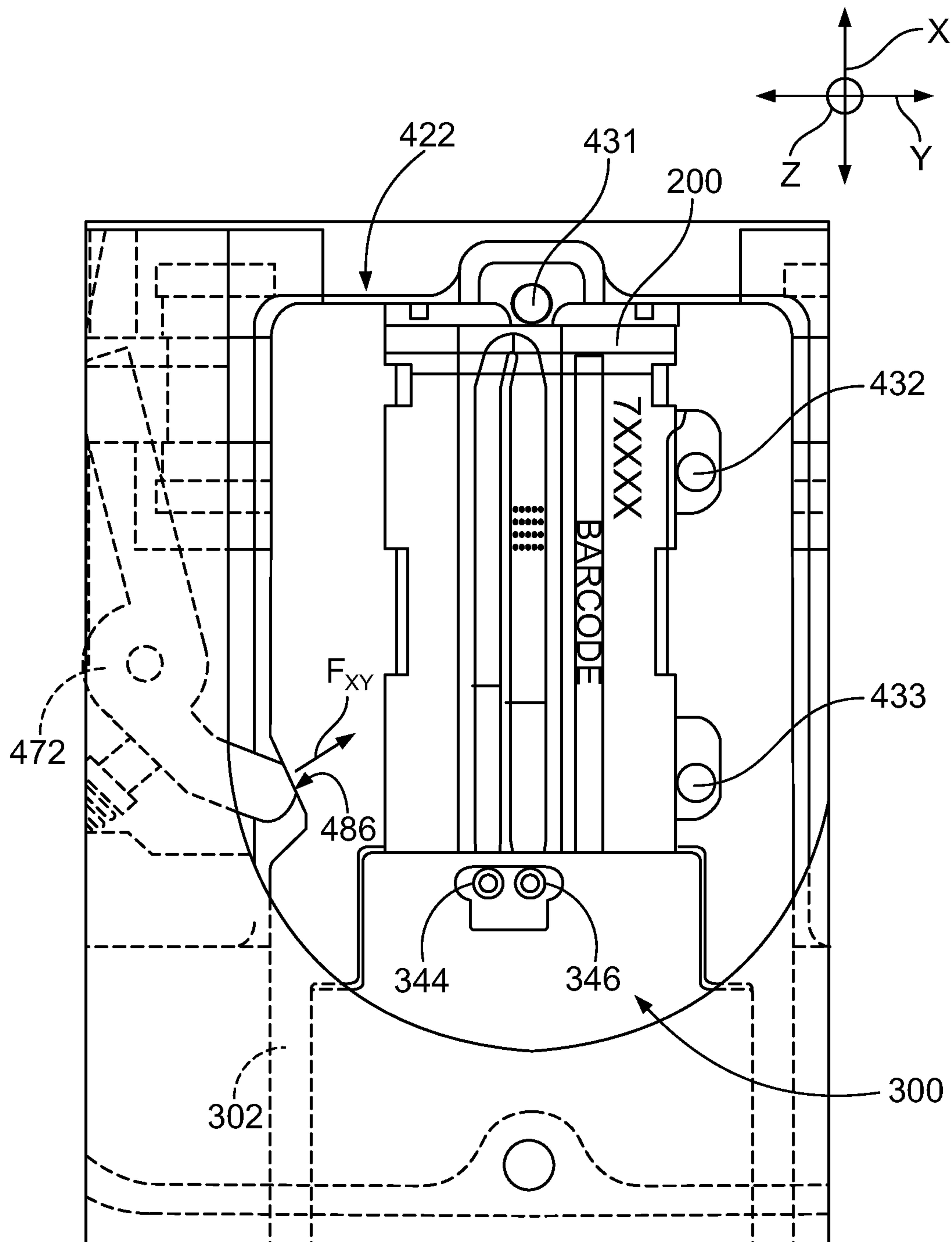


FIG. 21



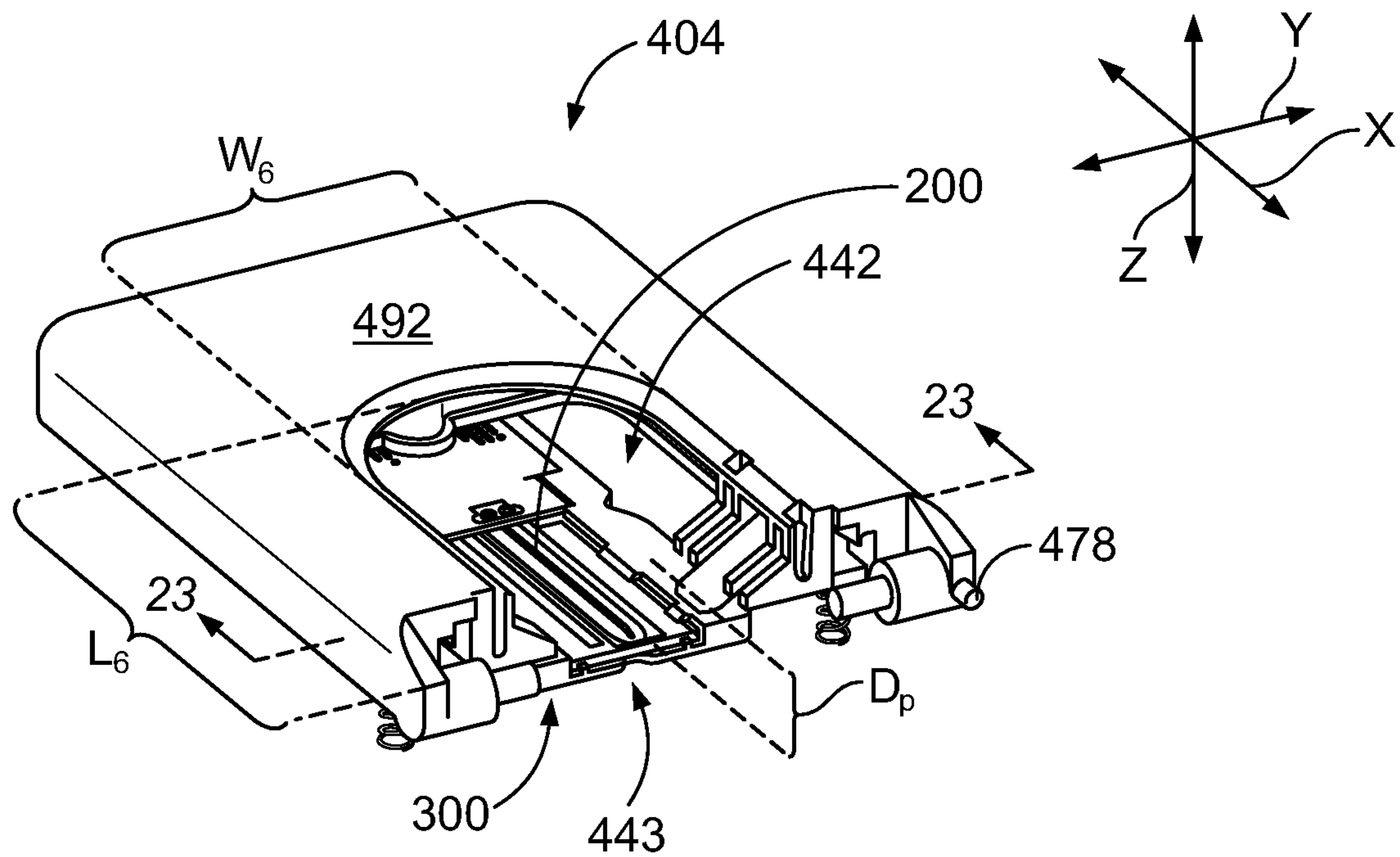


FIG. 22

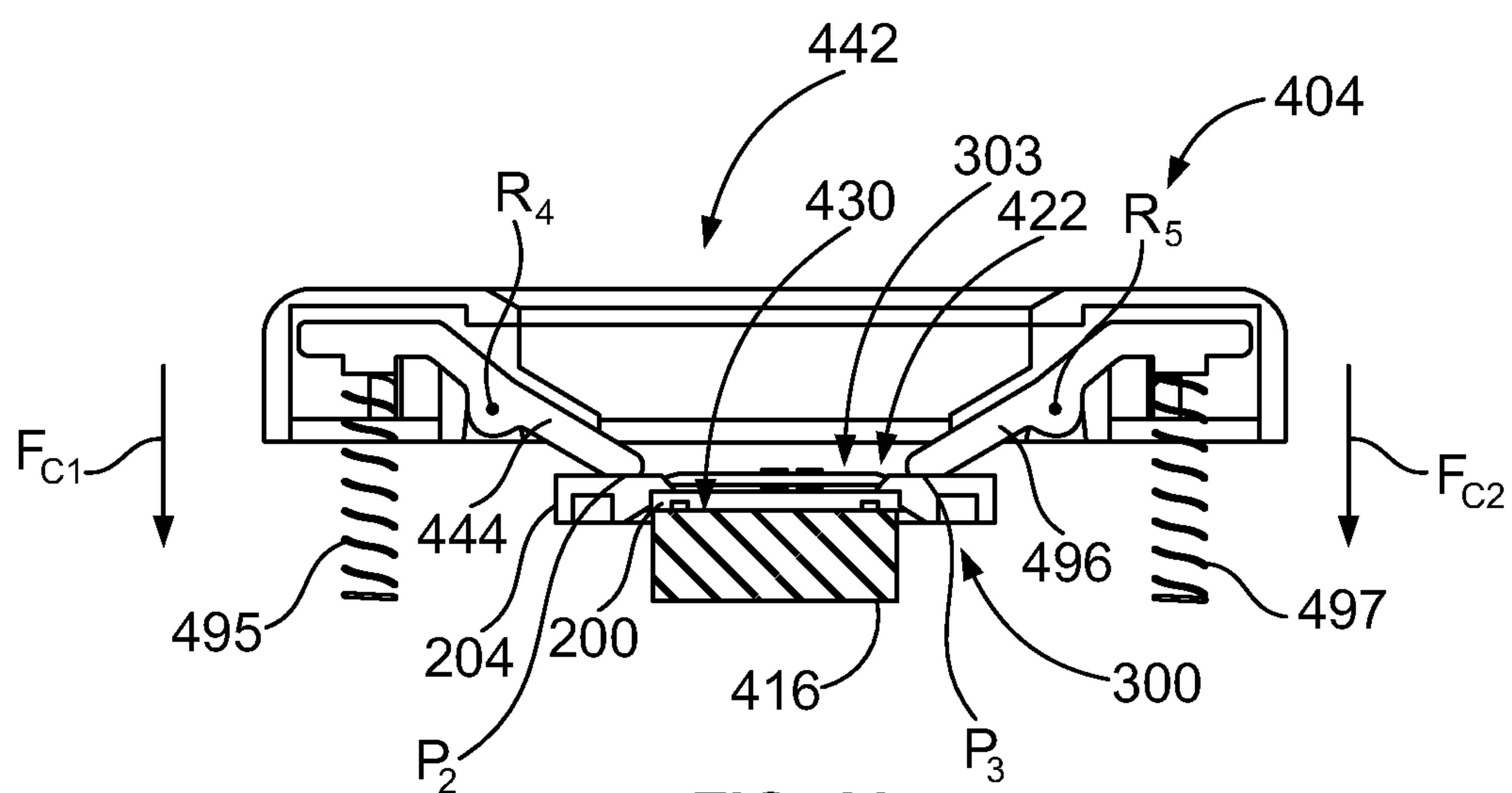


FIG. 23



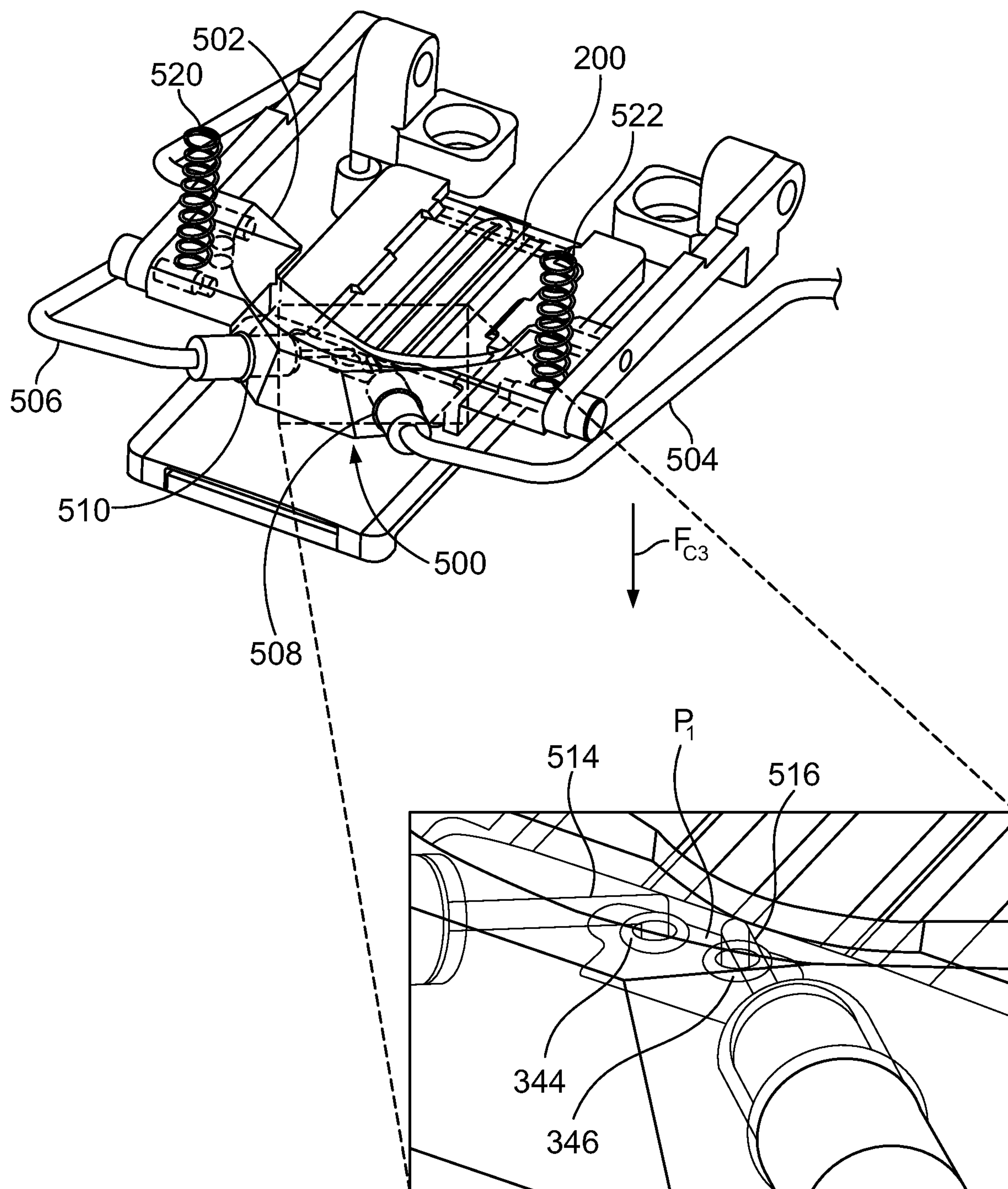
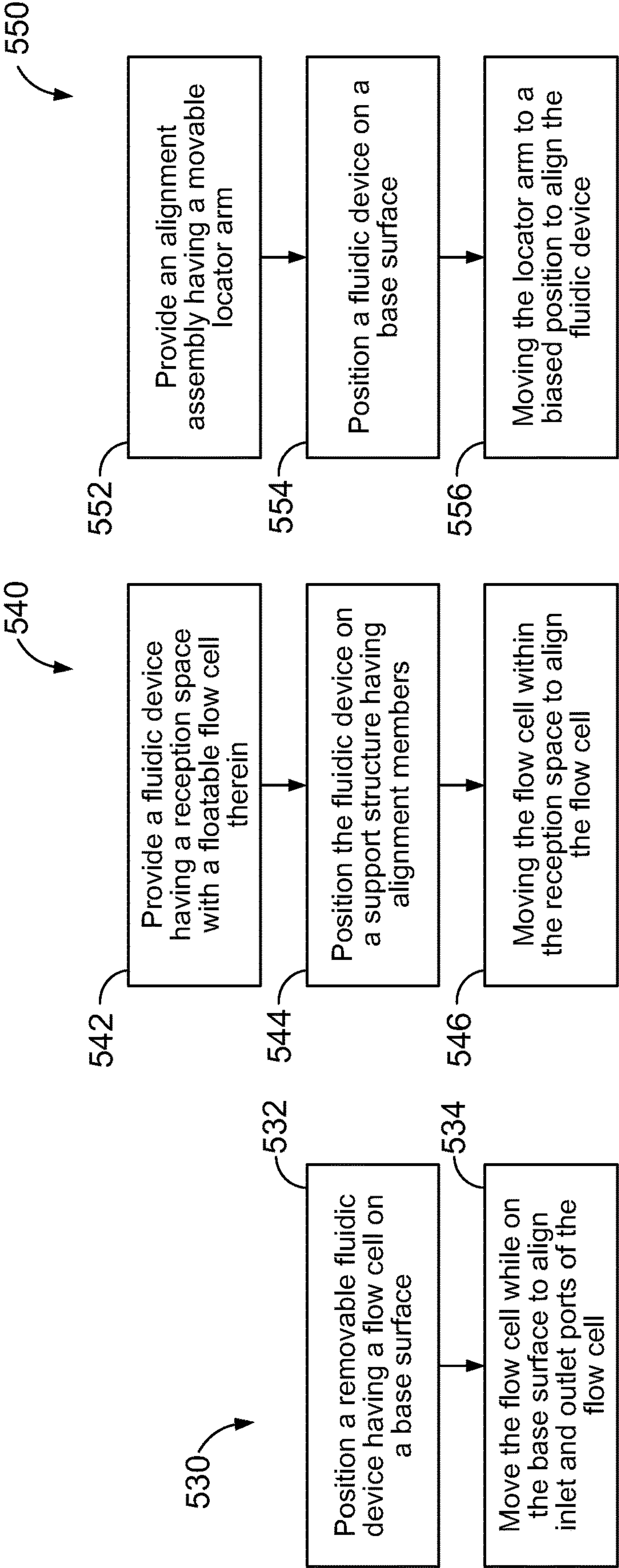


FIG. 24







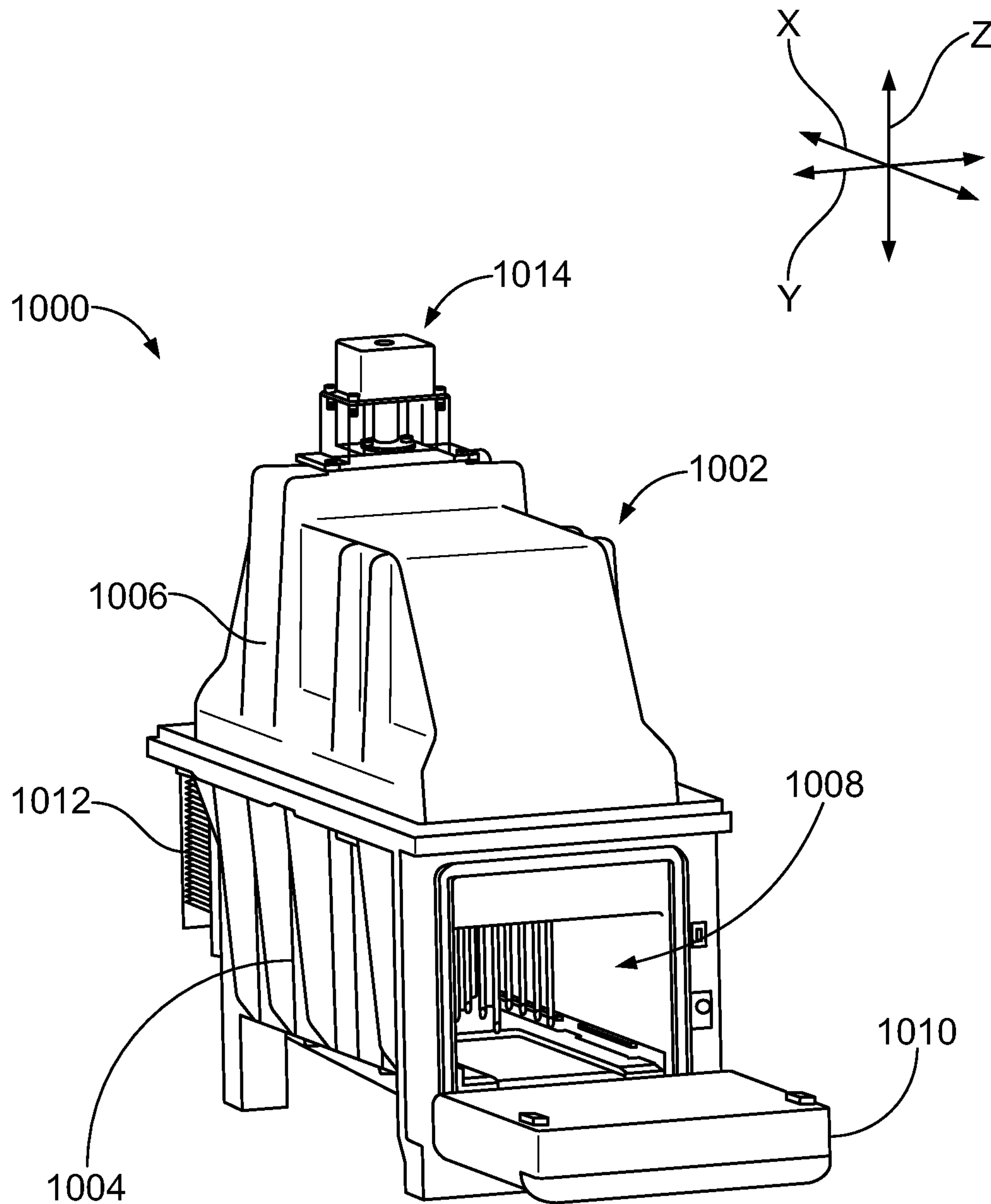


FIG. 28



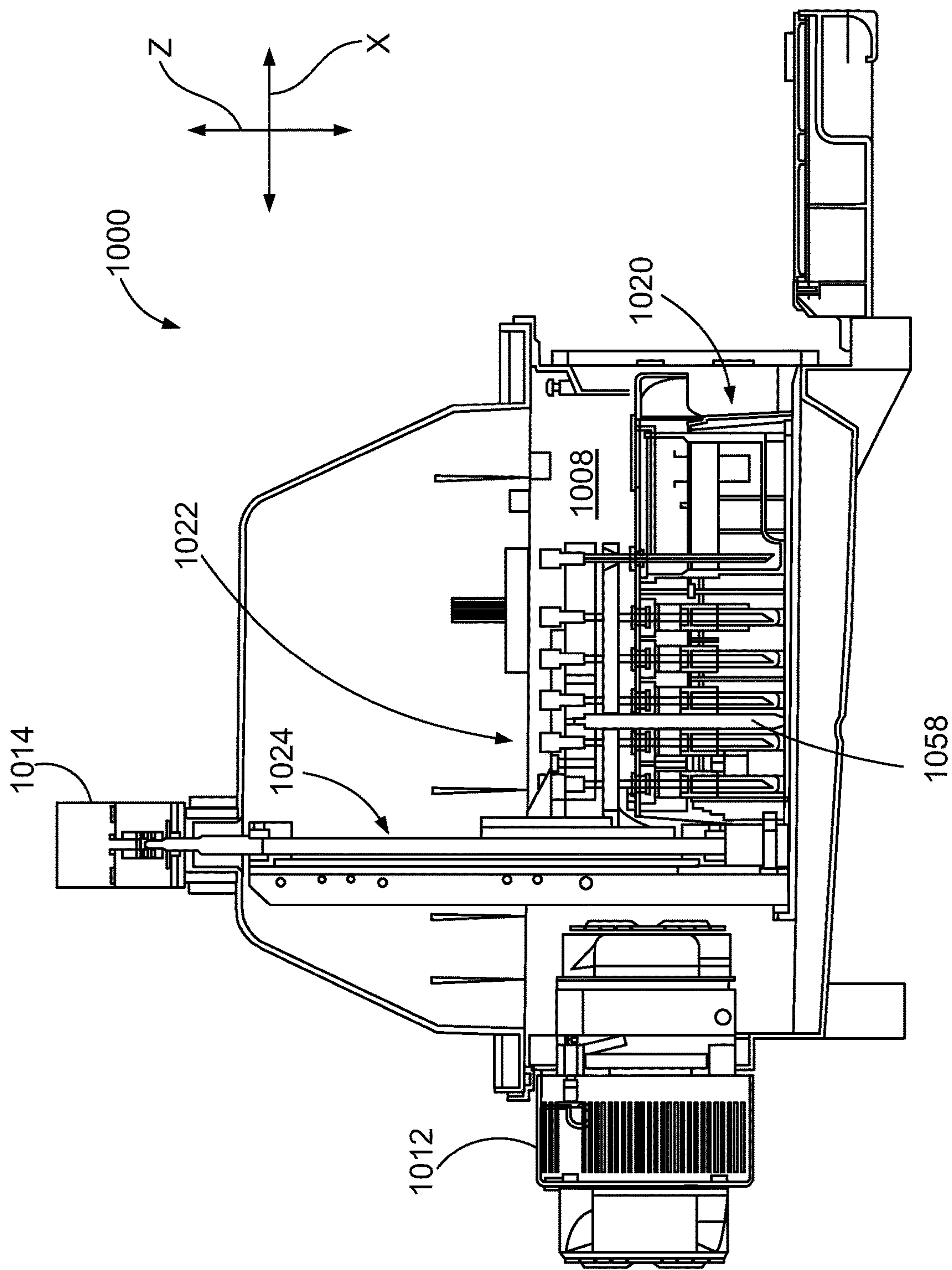


FIG. 29







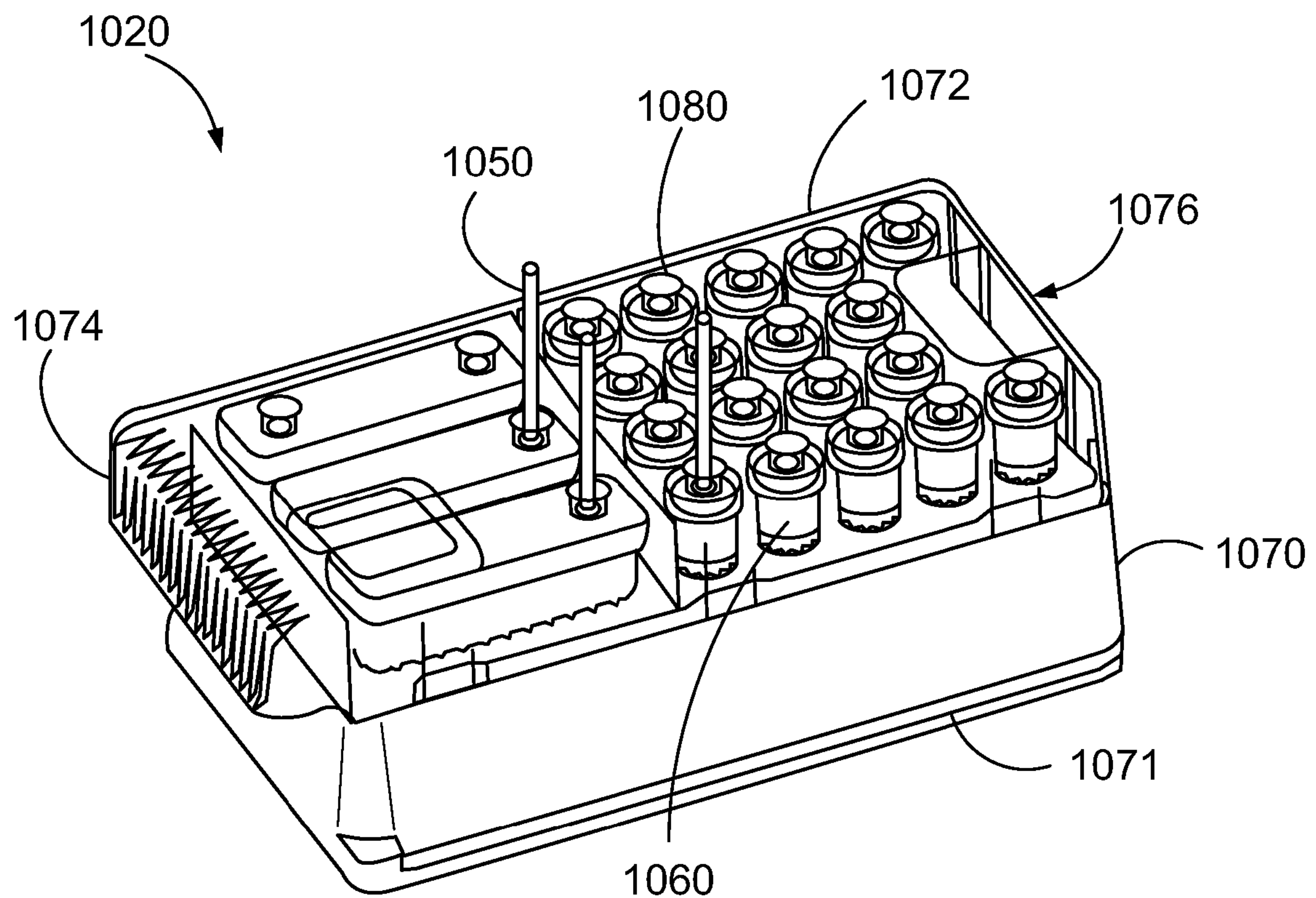


FIG. 31

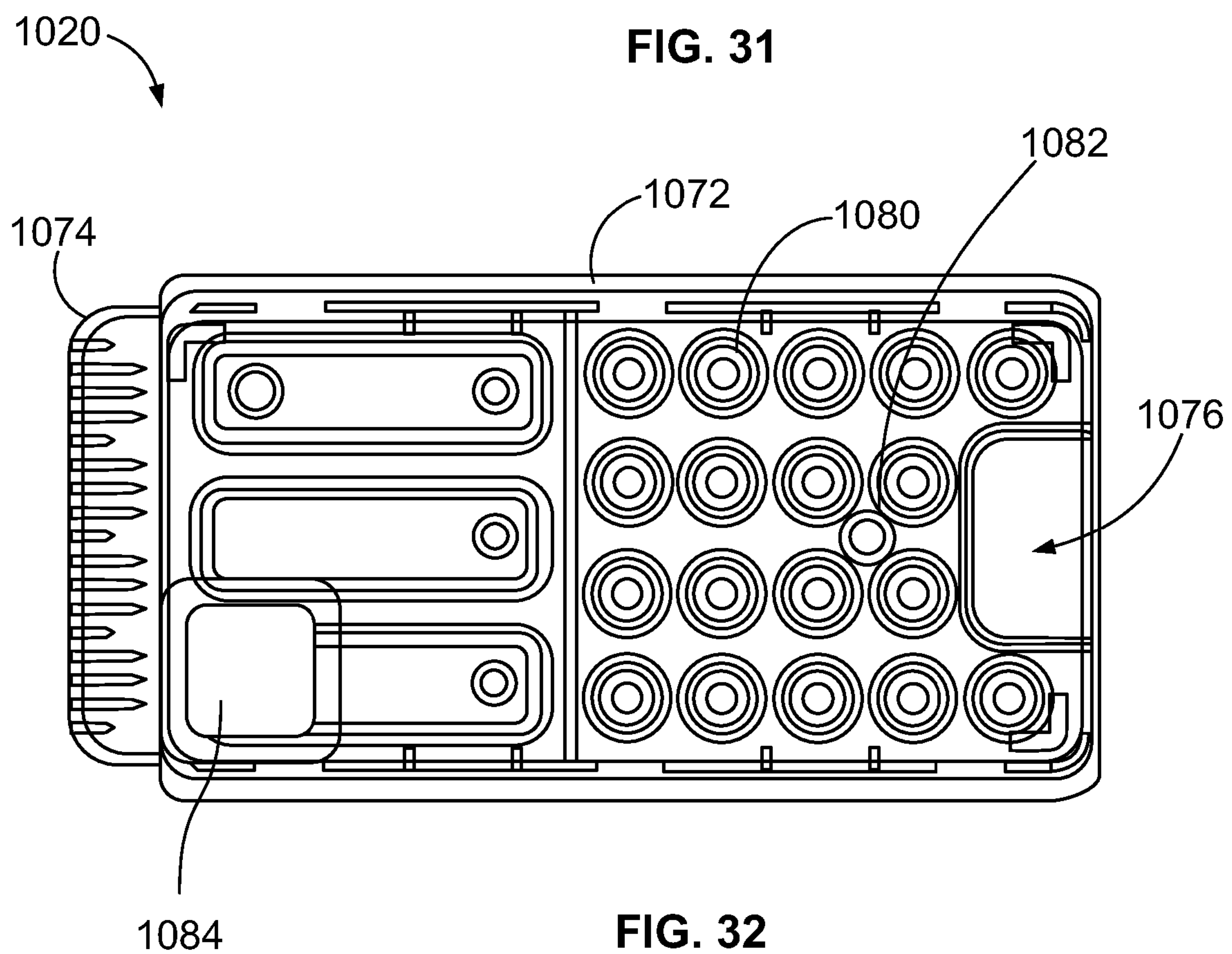


FIG. 32



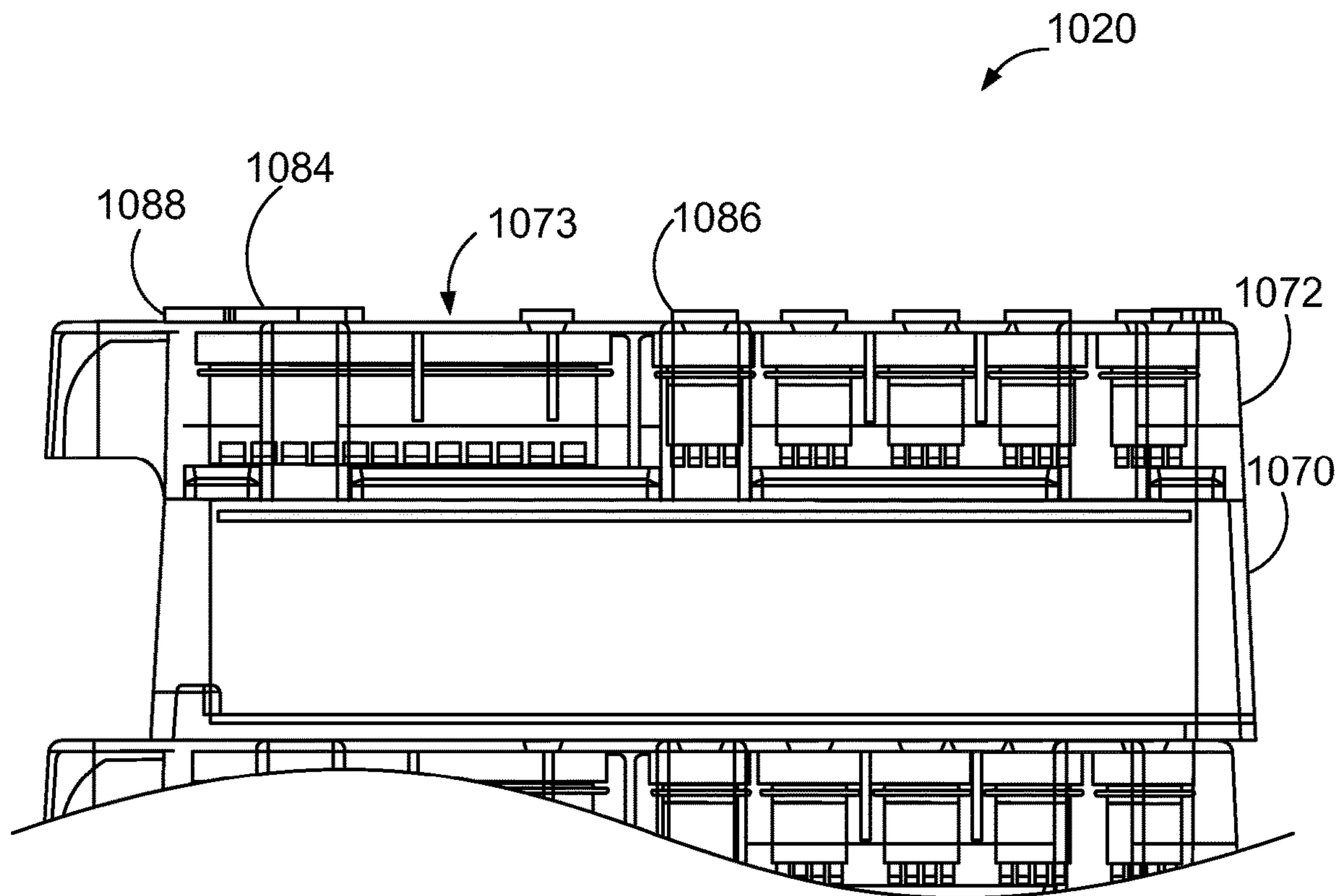


FIG. 33

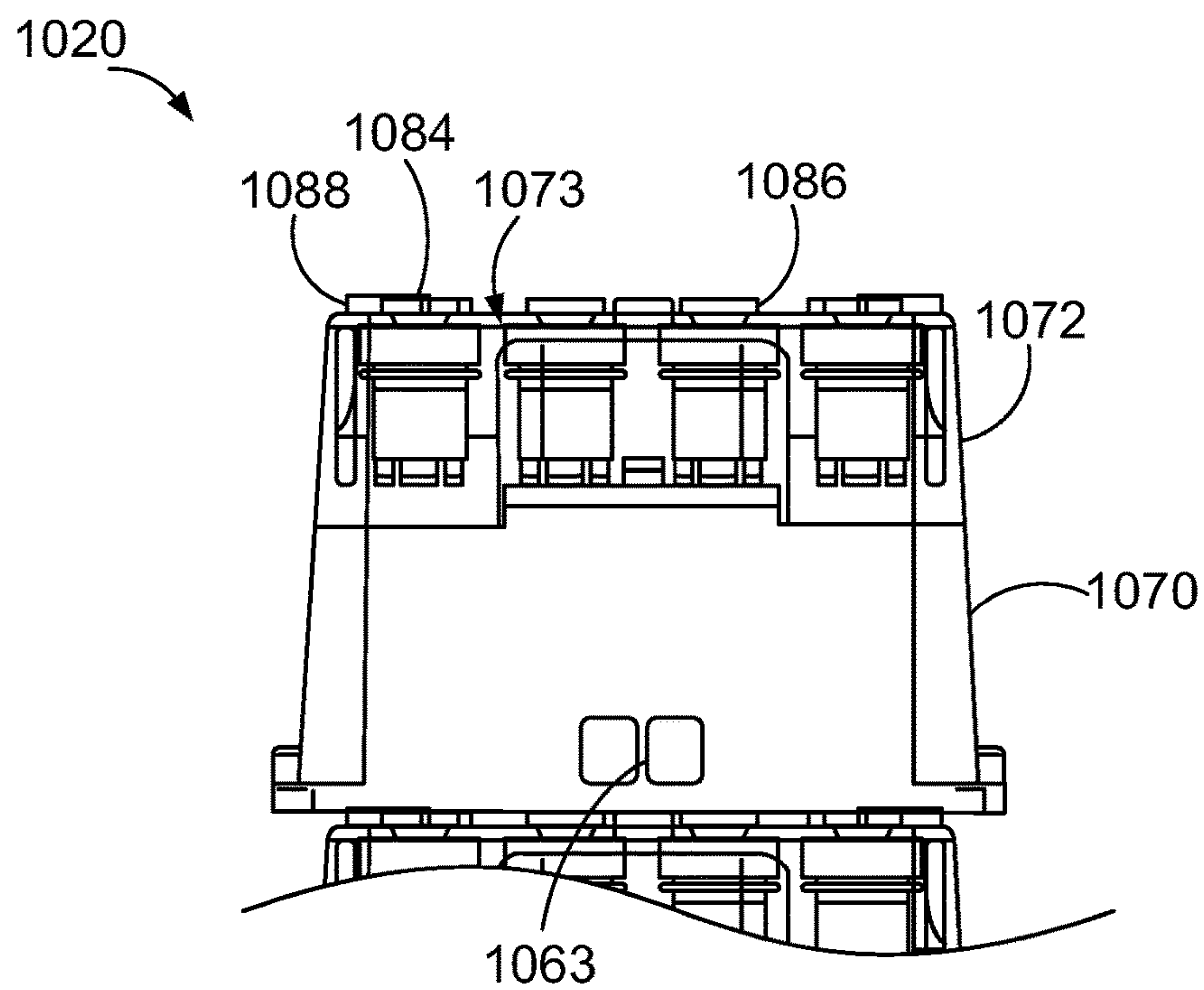


FIG. 34



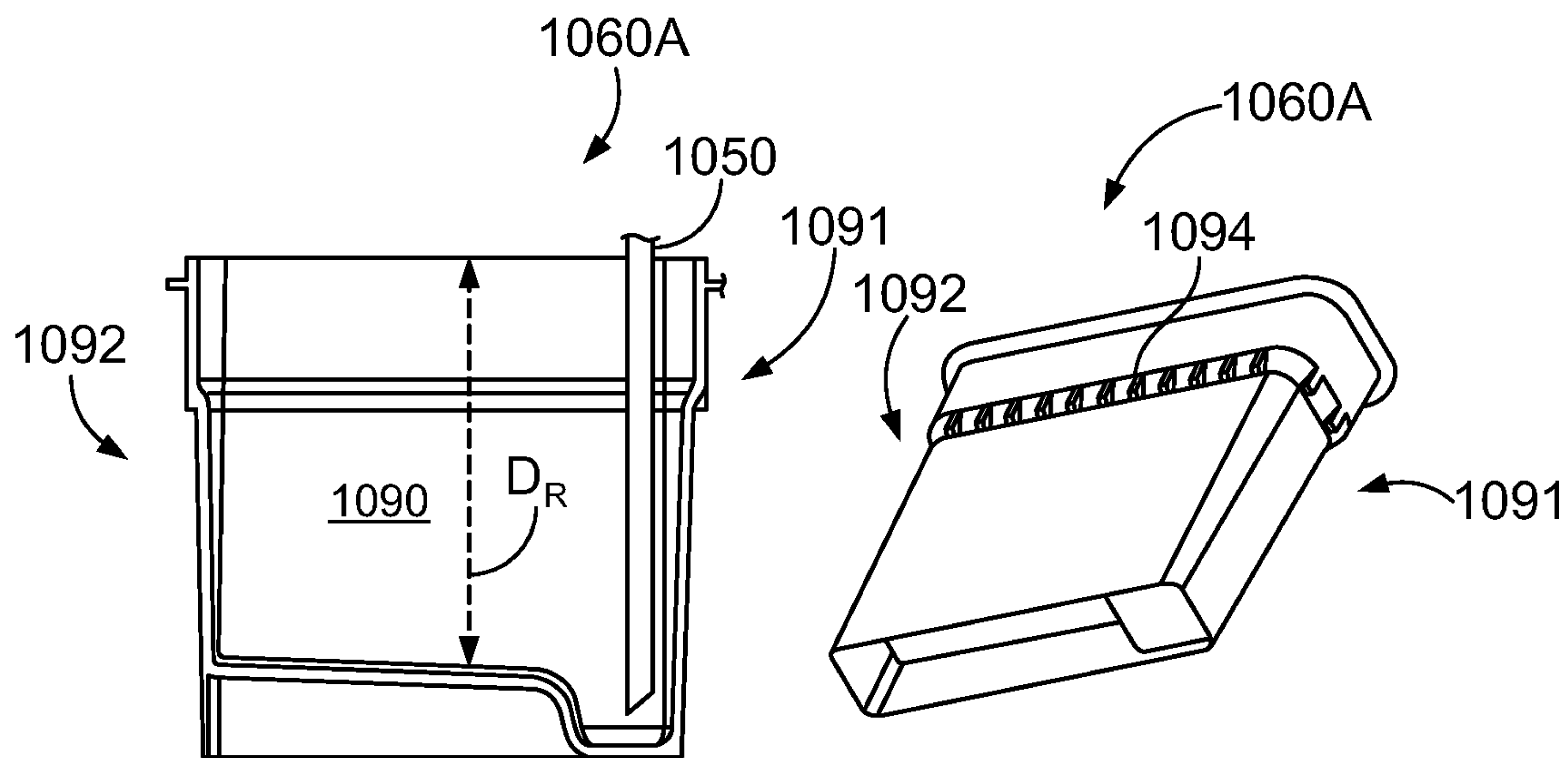


FIG. 36

FIG. 35

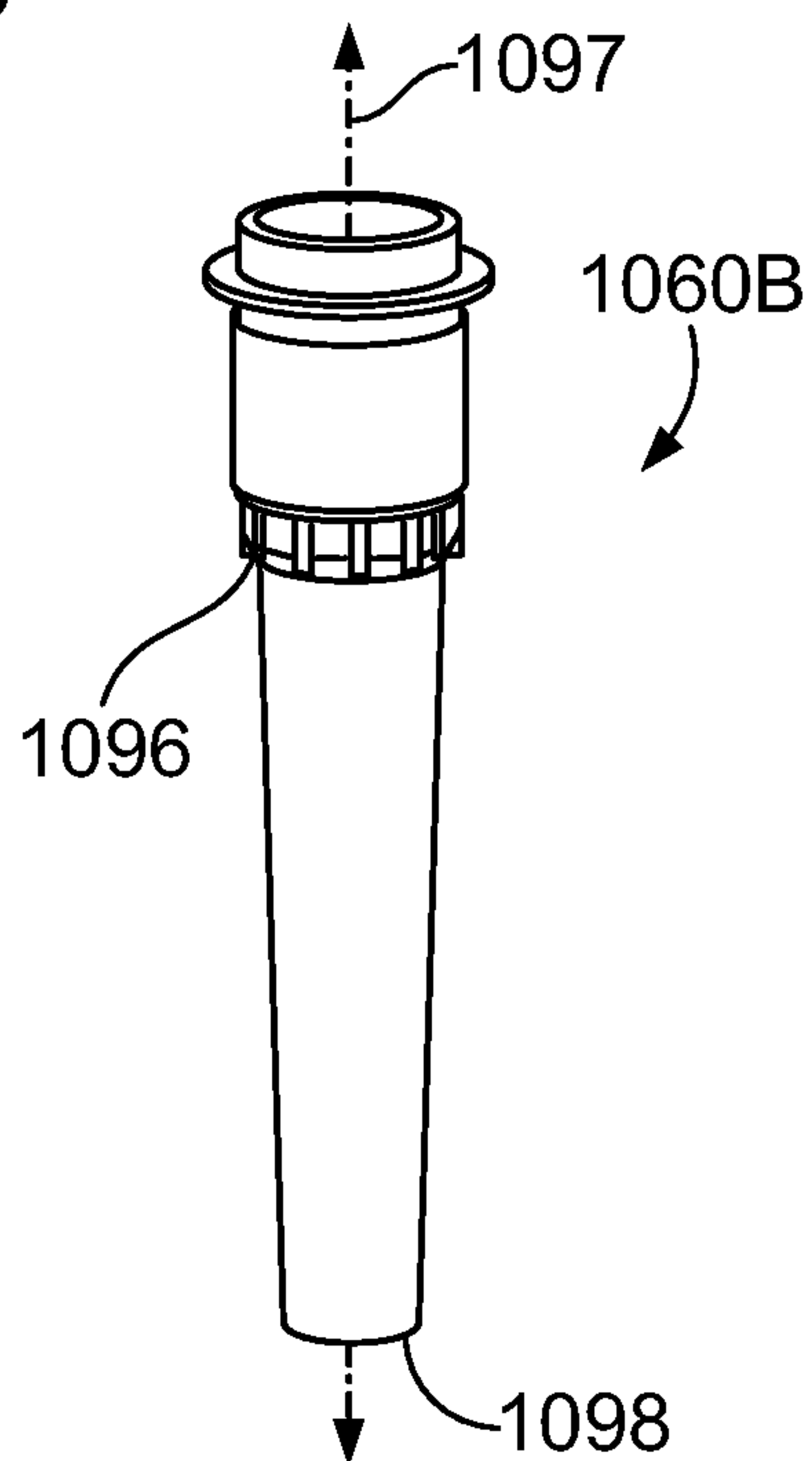


FIG. 37





**FIG. 38**



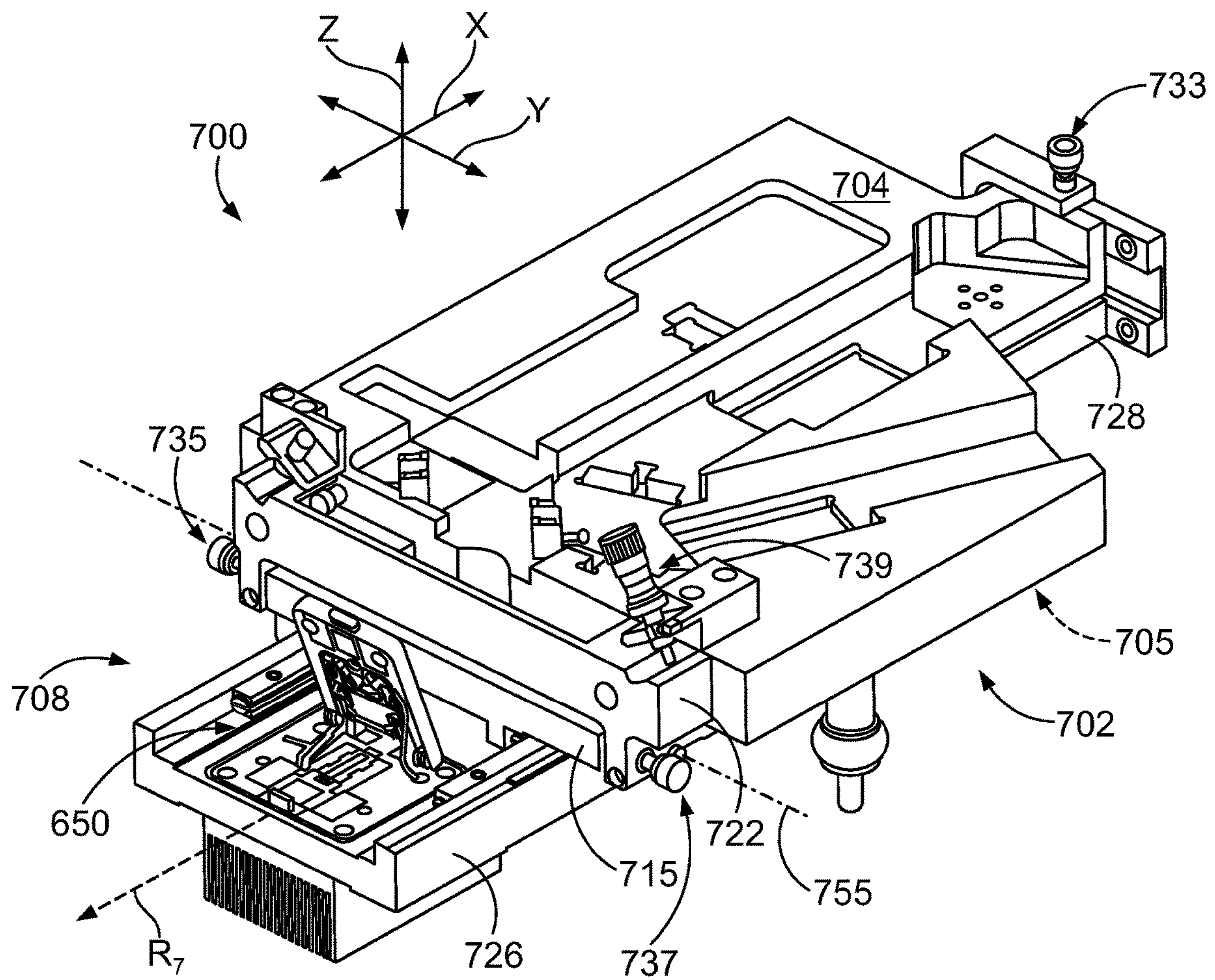


FIG. 39



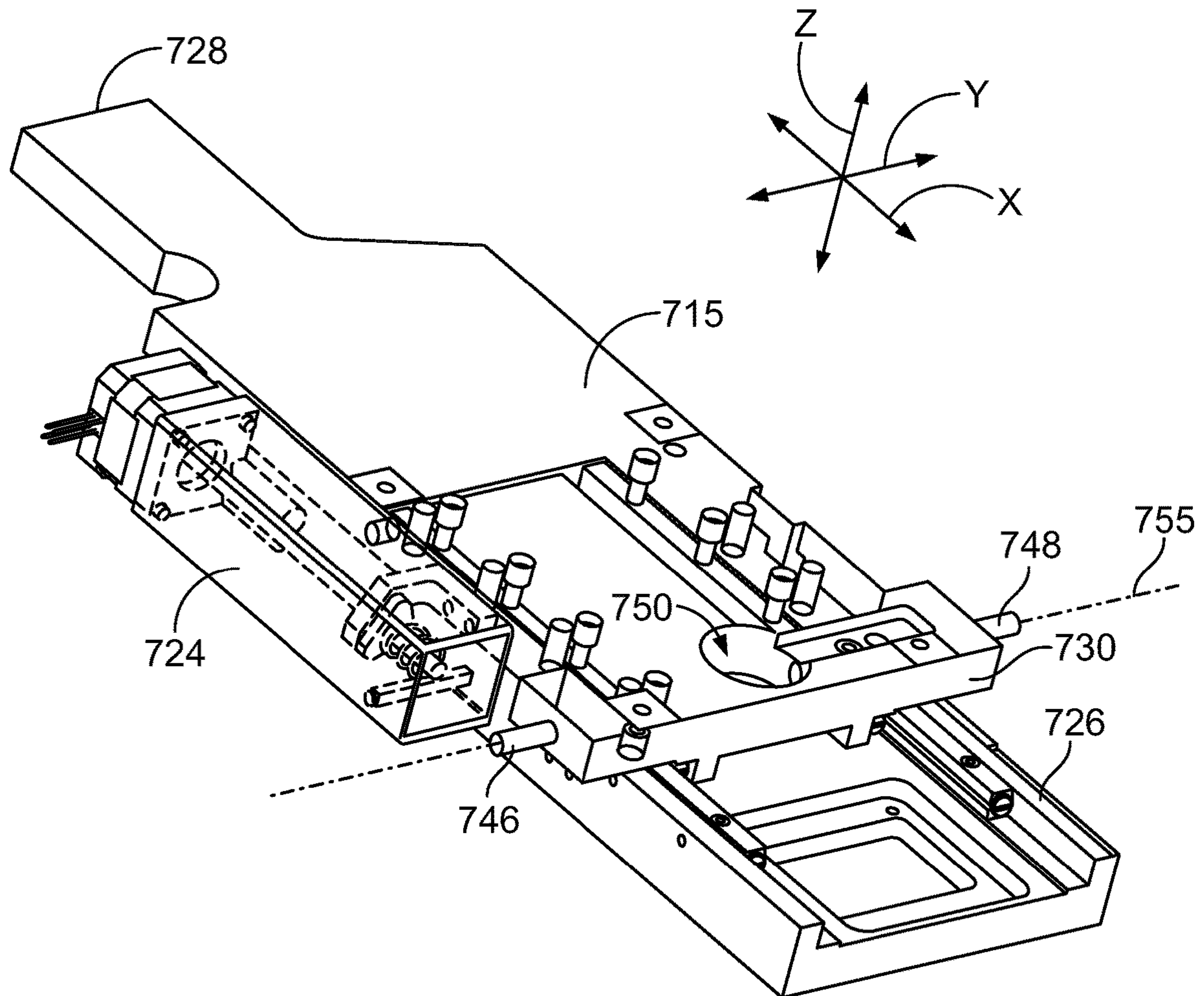
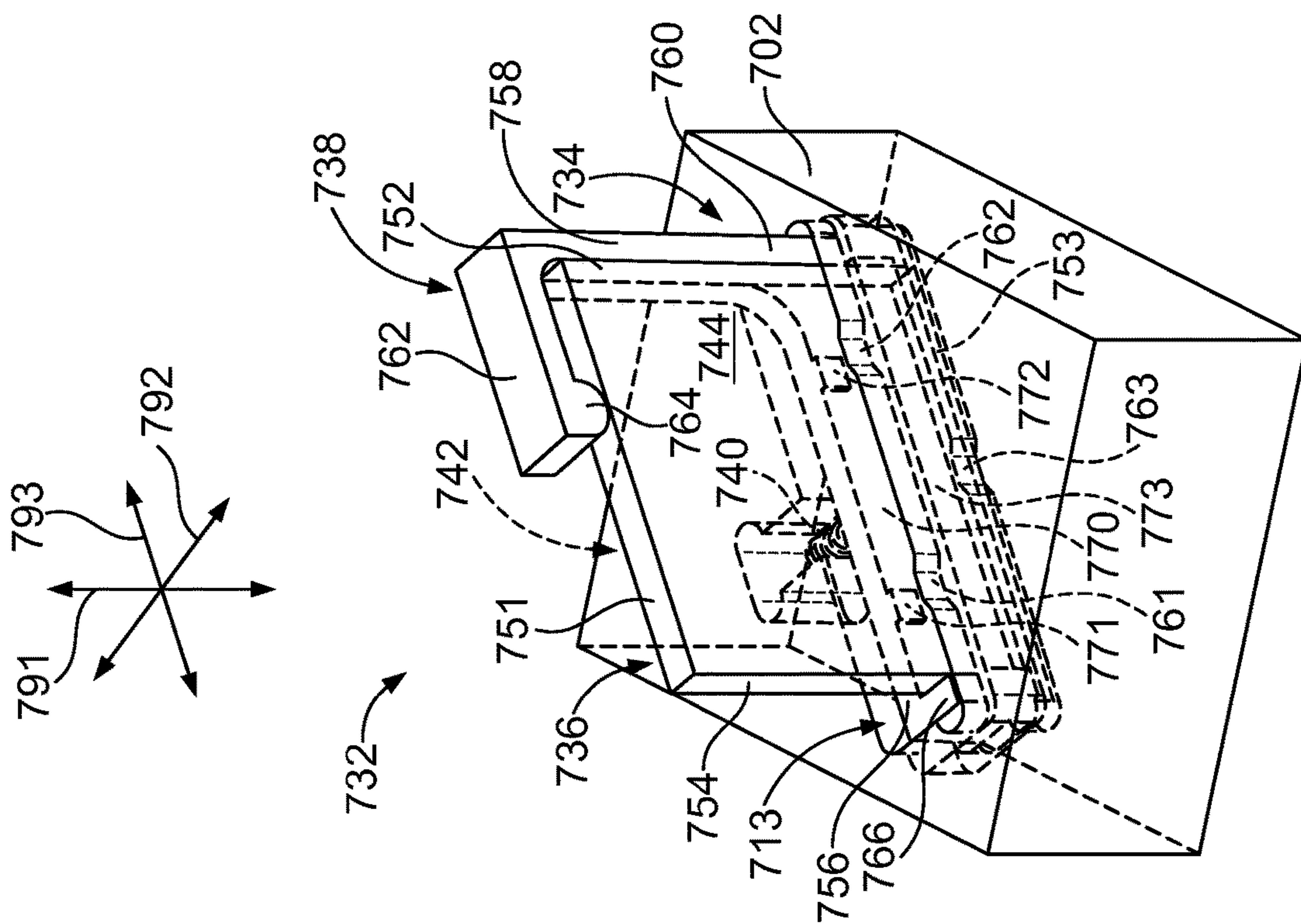


FIG. 40

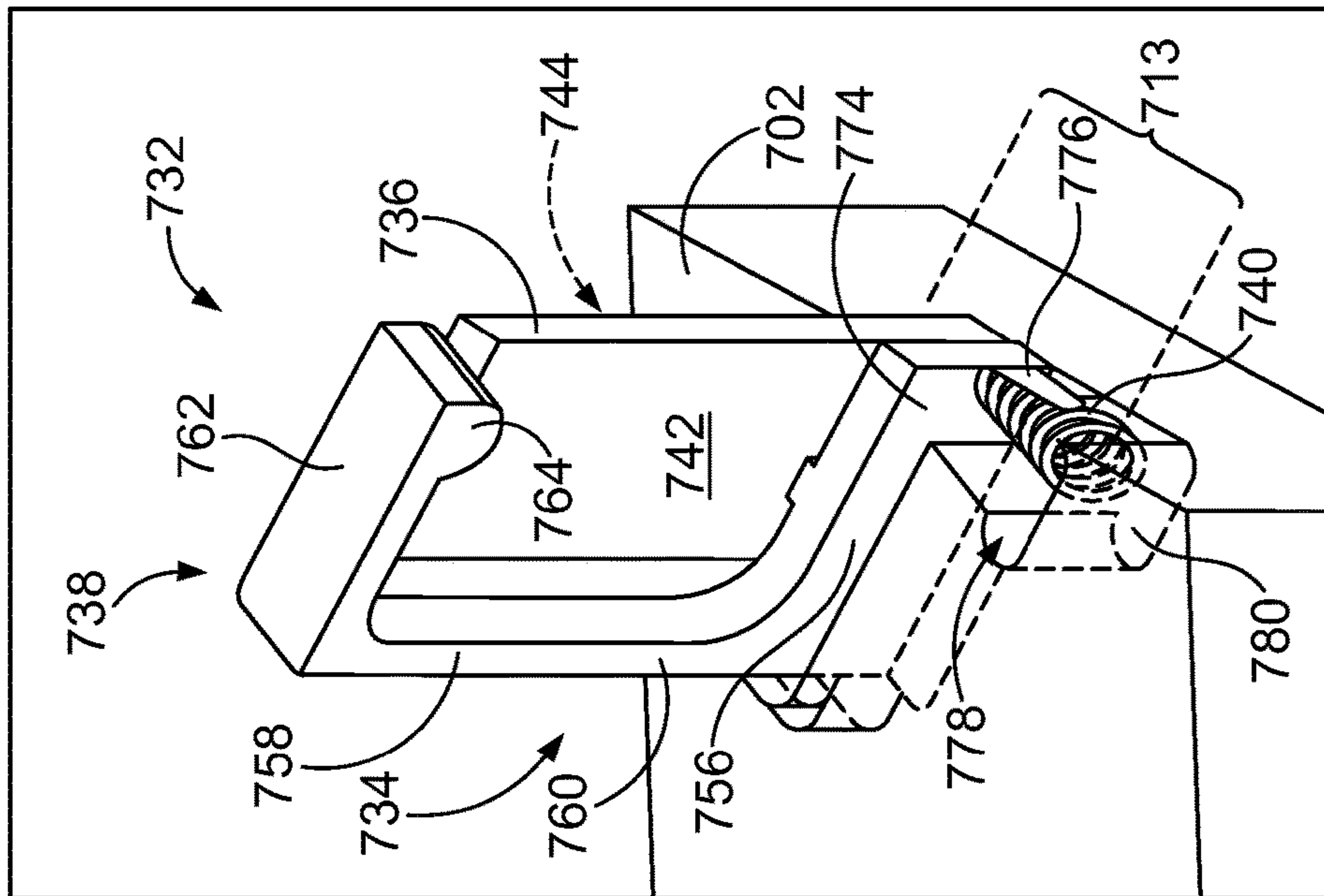






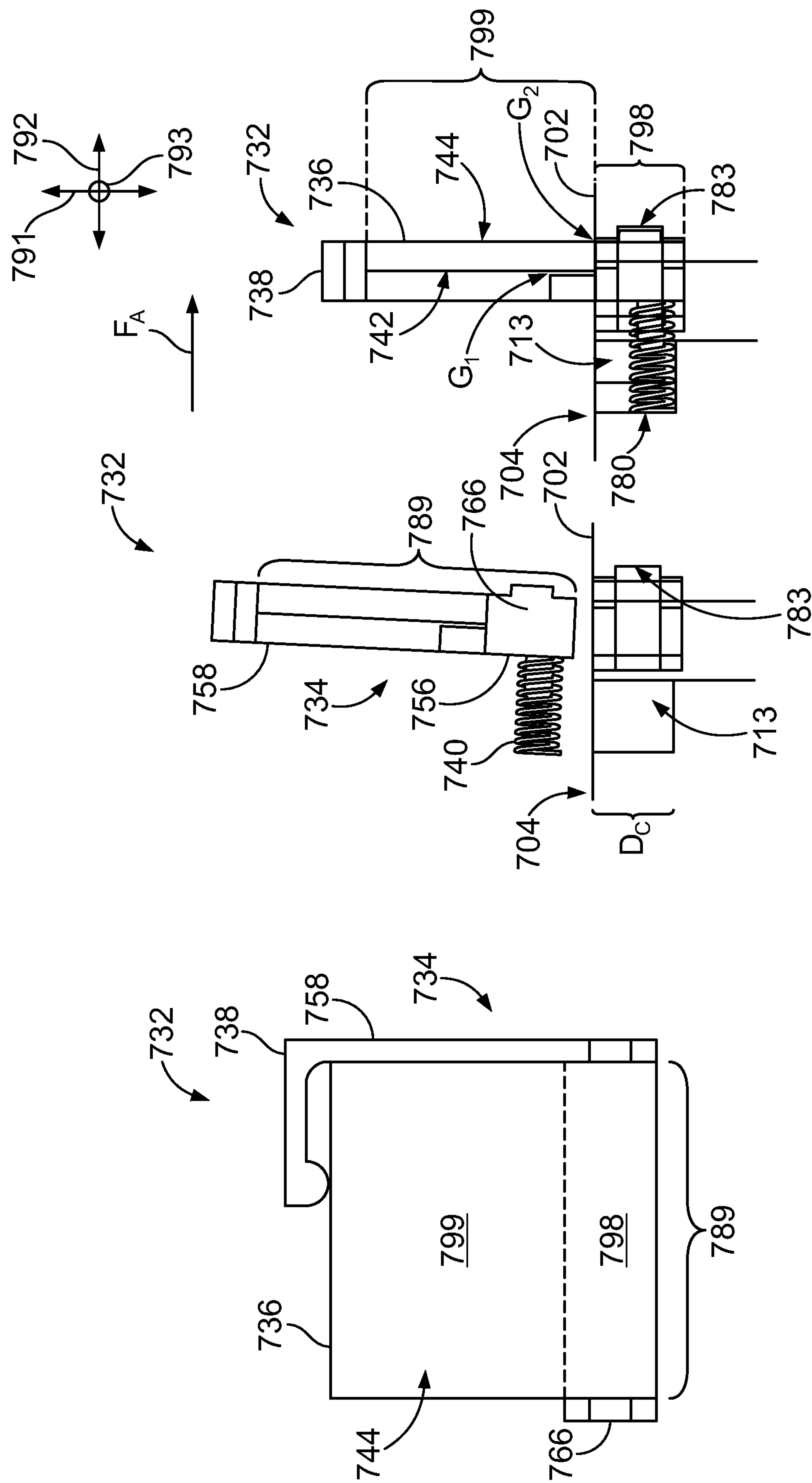


**FIG. 43**



**FIG. 44**

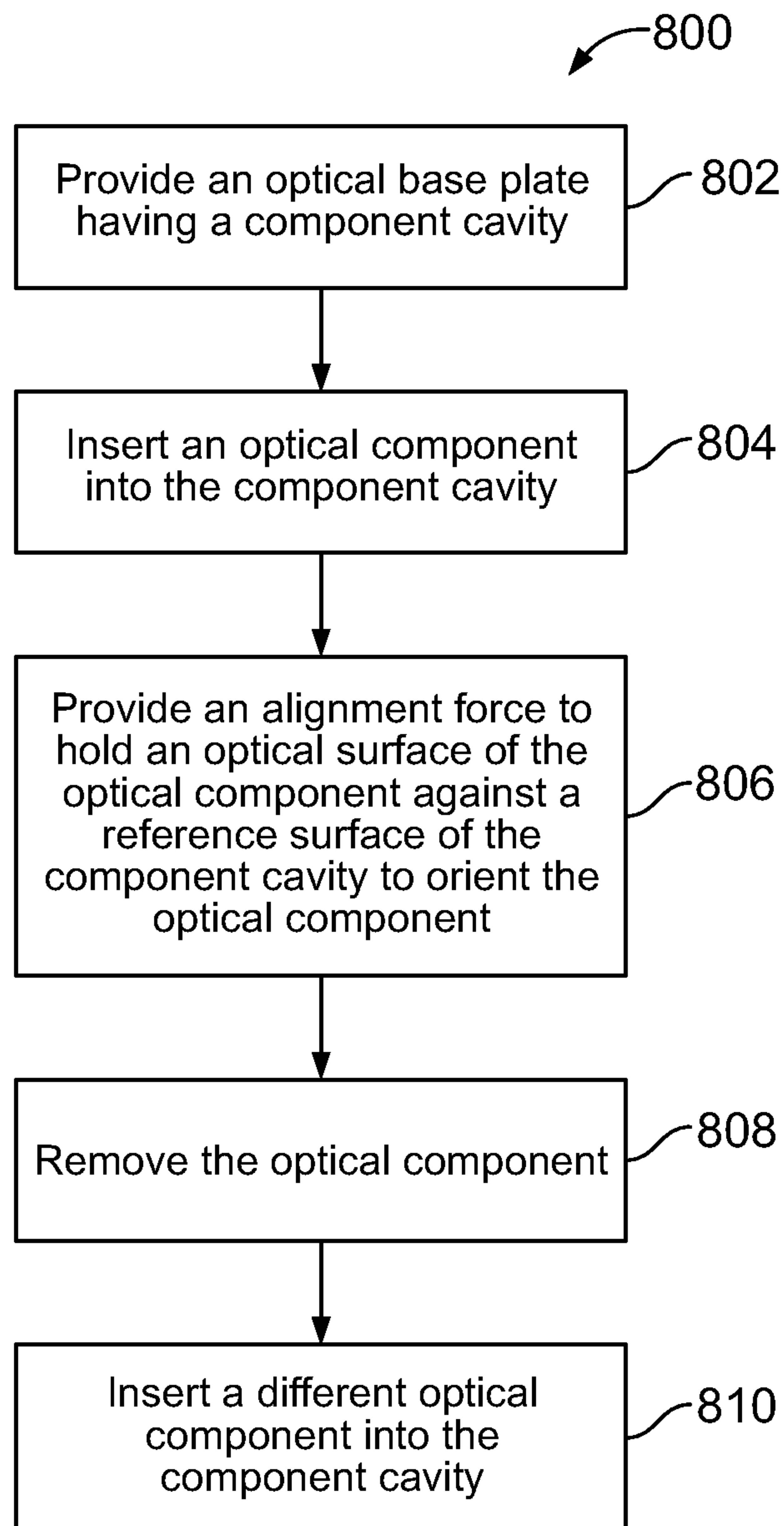




**FIG. 45**

**FIG. 46**



**FIG. 47**



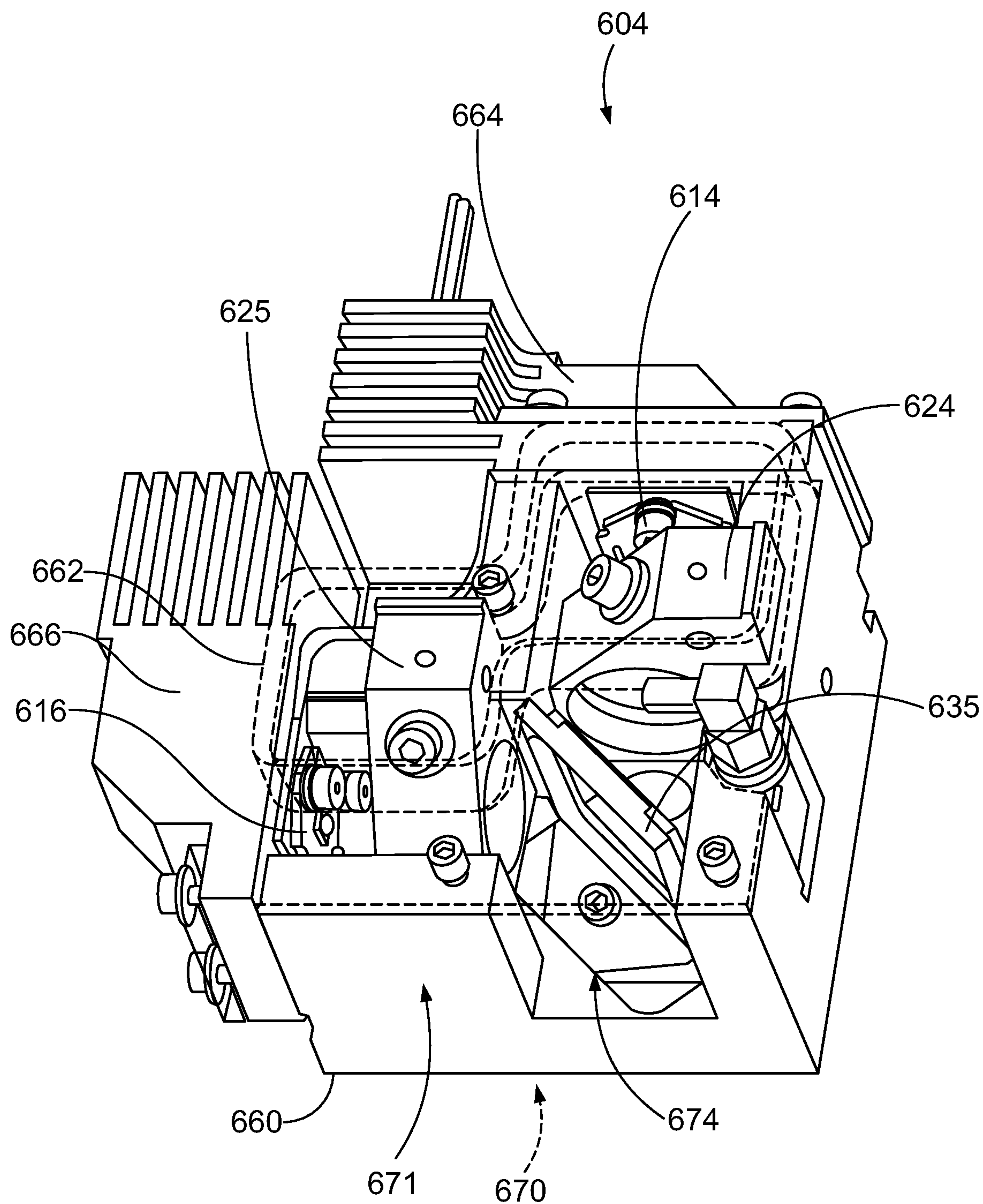


FIG.48



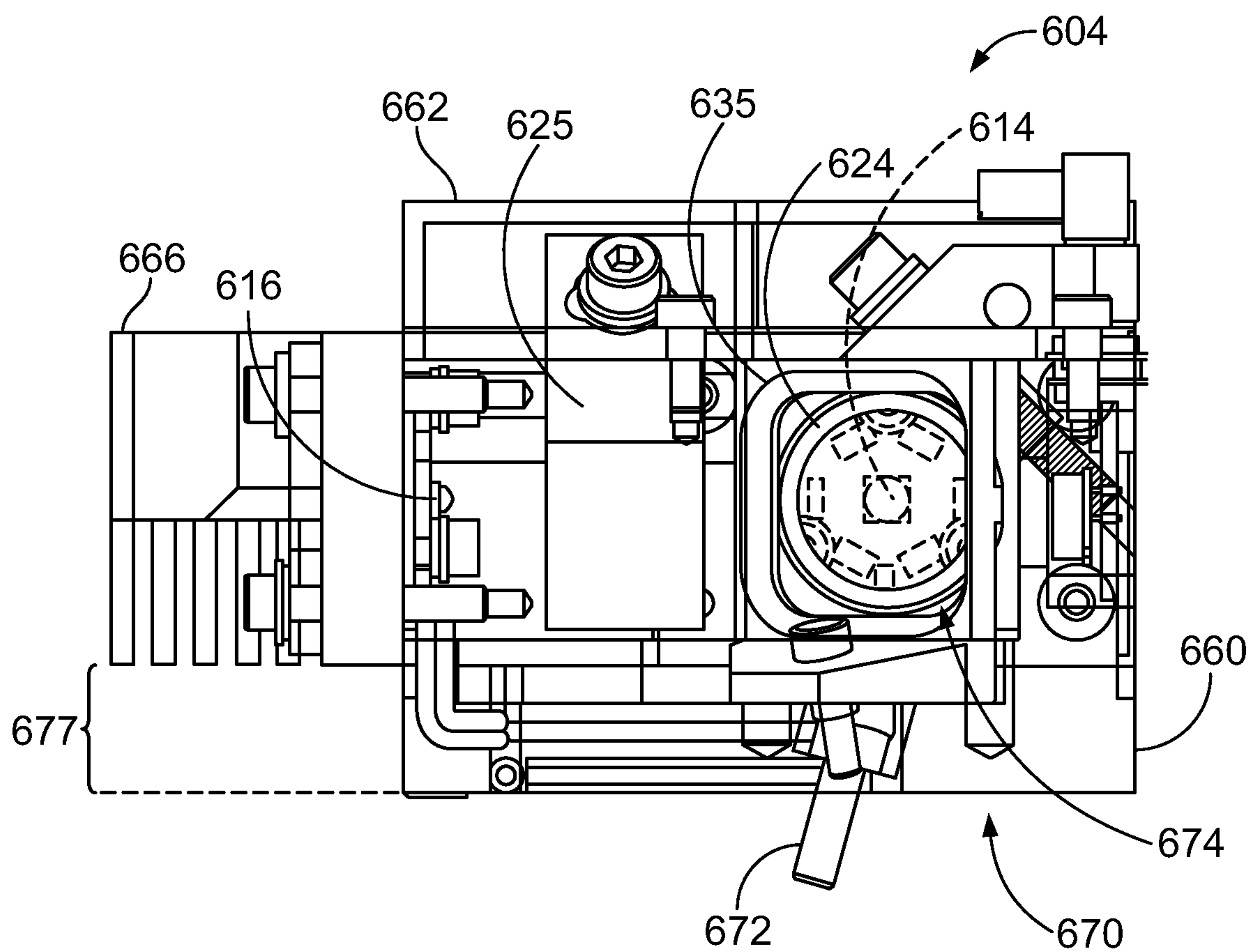


FIG. 49



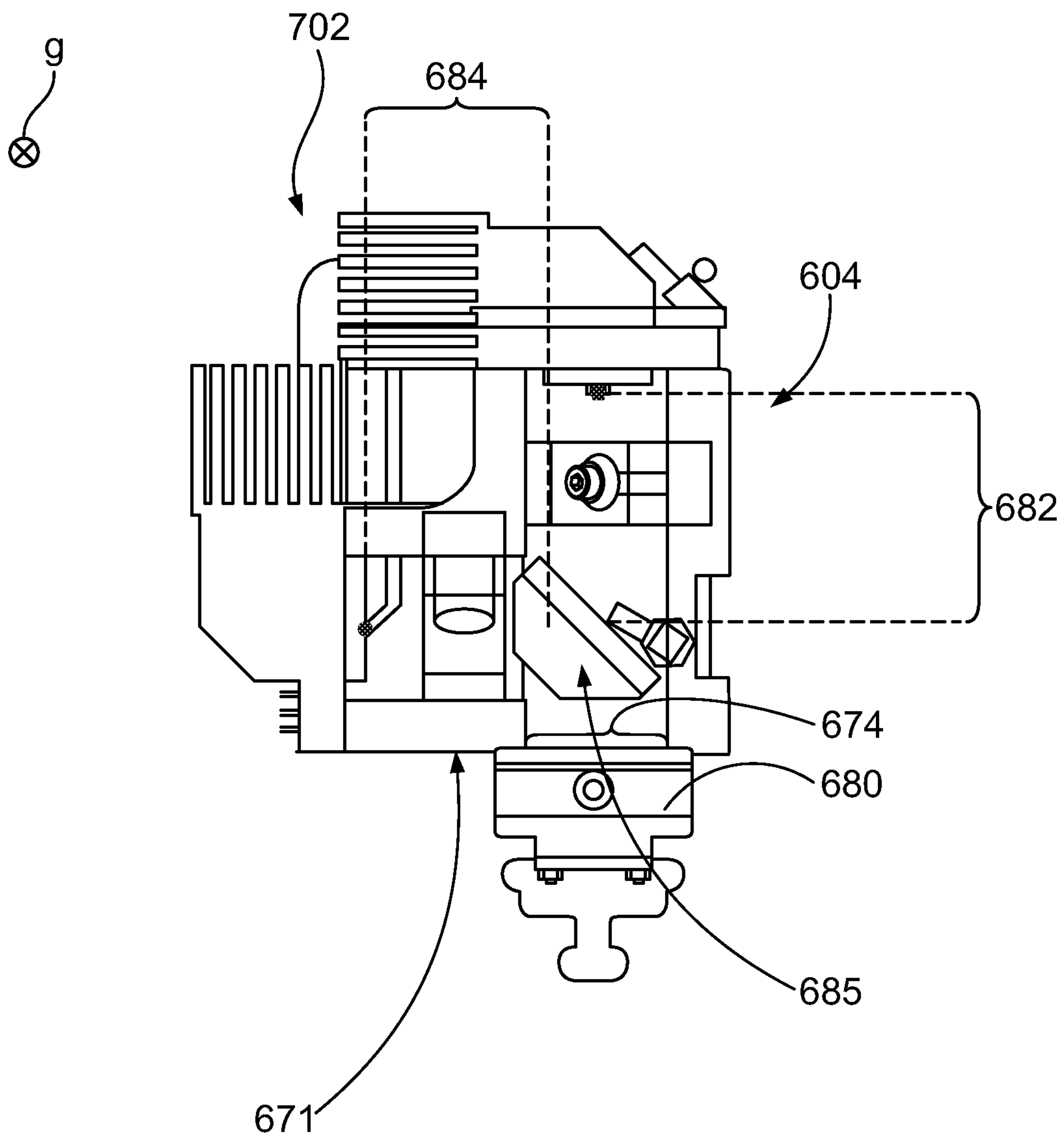


FIG. 50







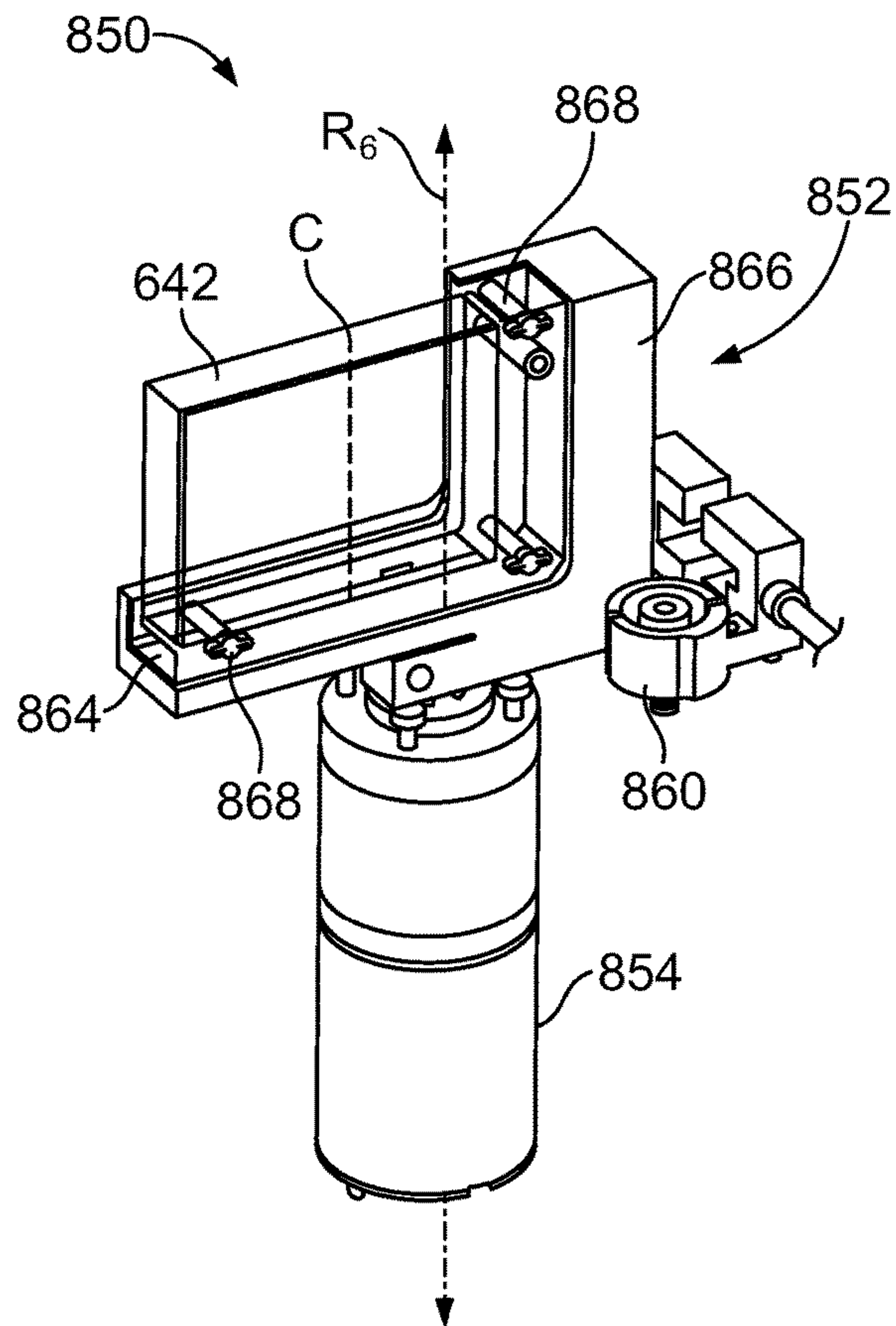


FIG. 52

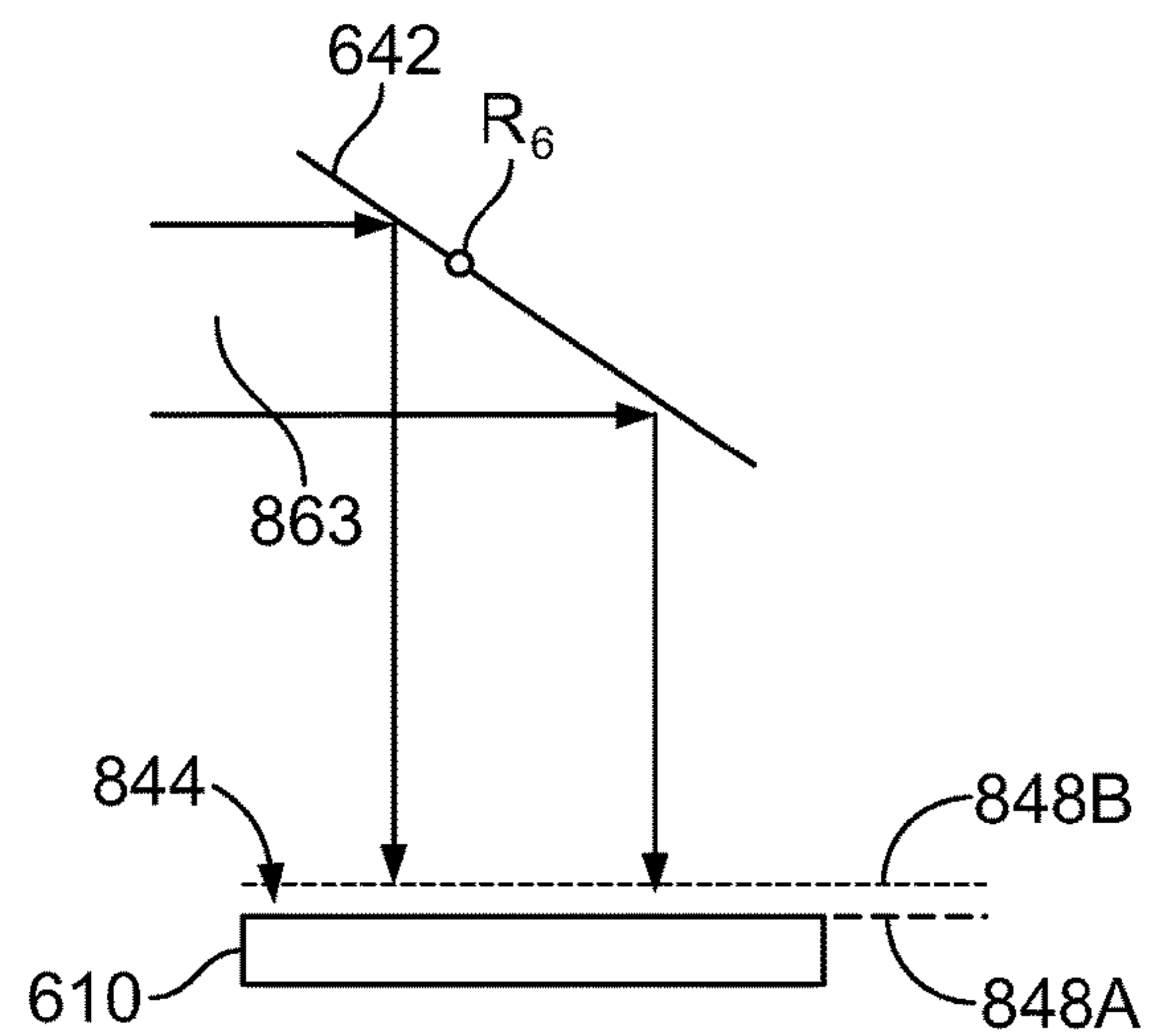


FIG. 53

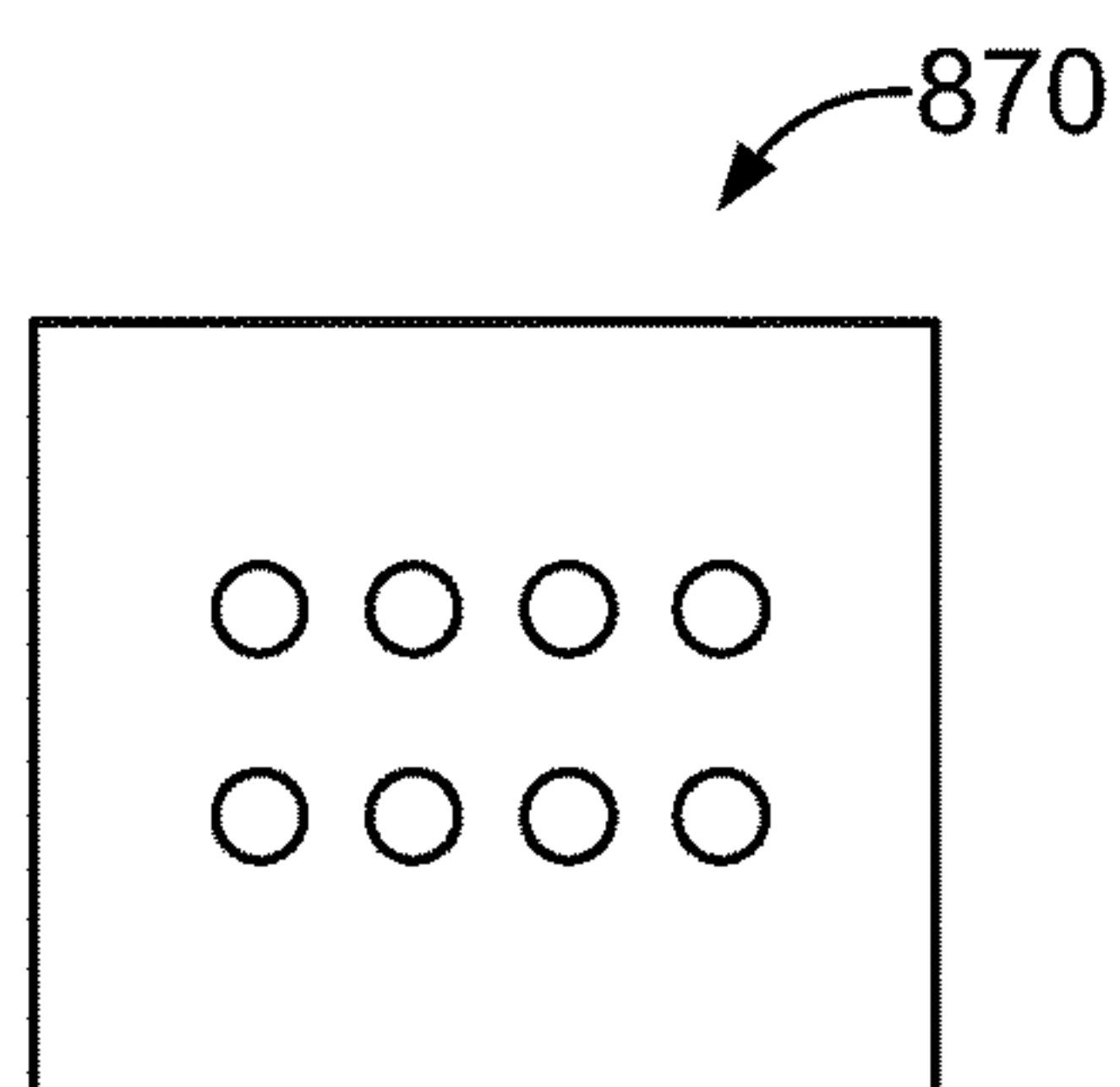


FIG. 54

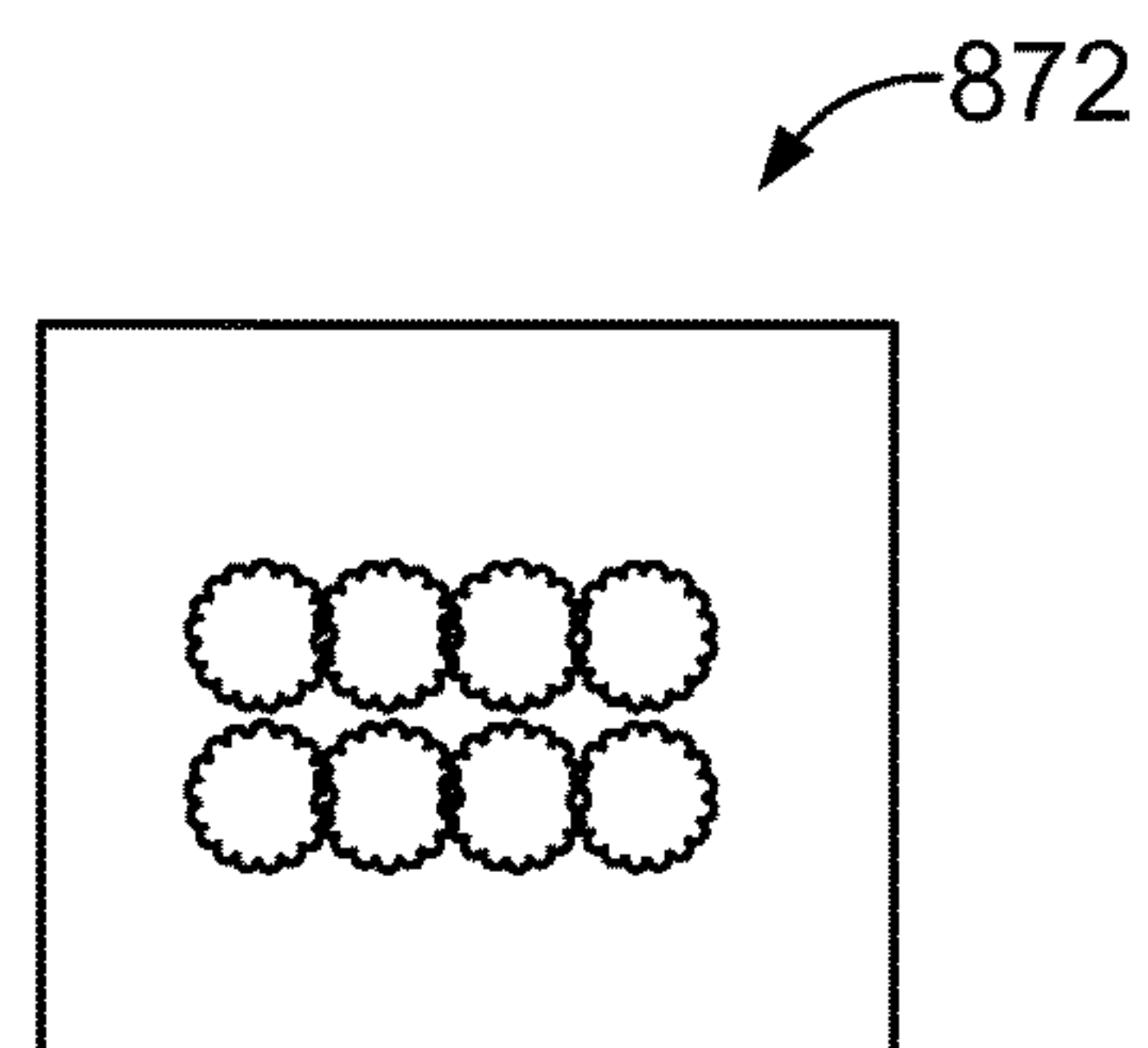


FIG. 55



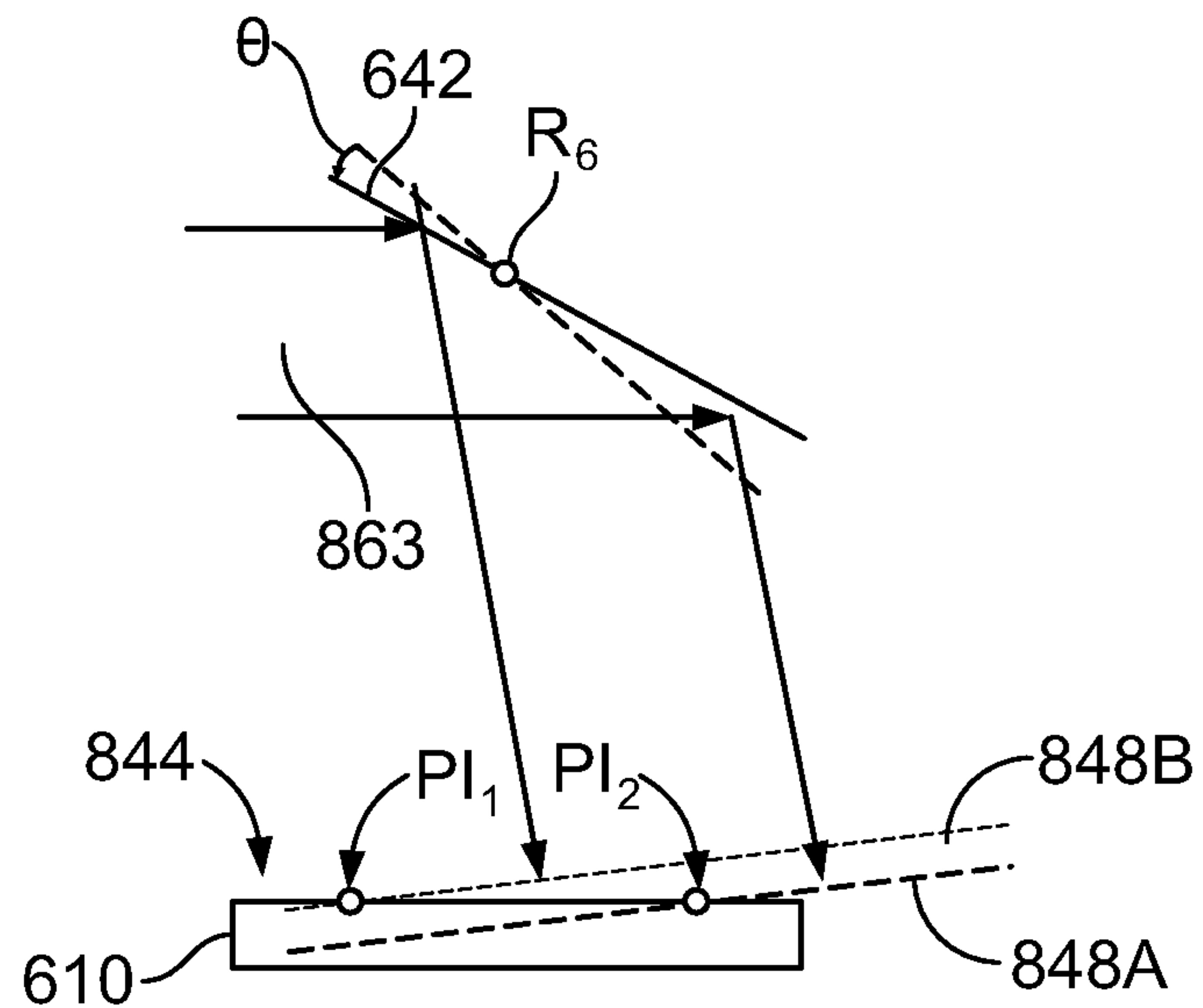


FIG. 56

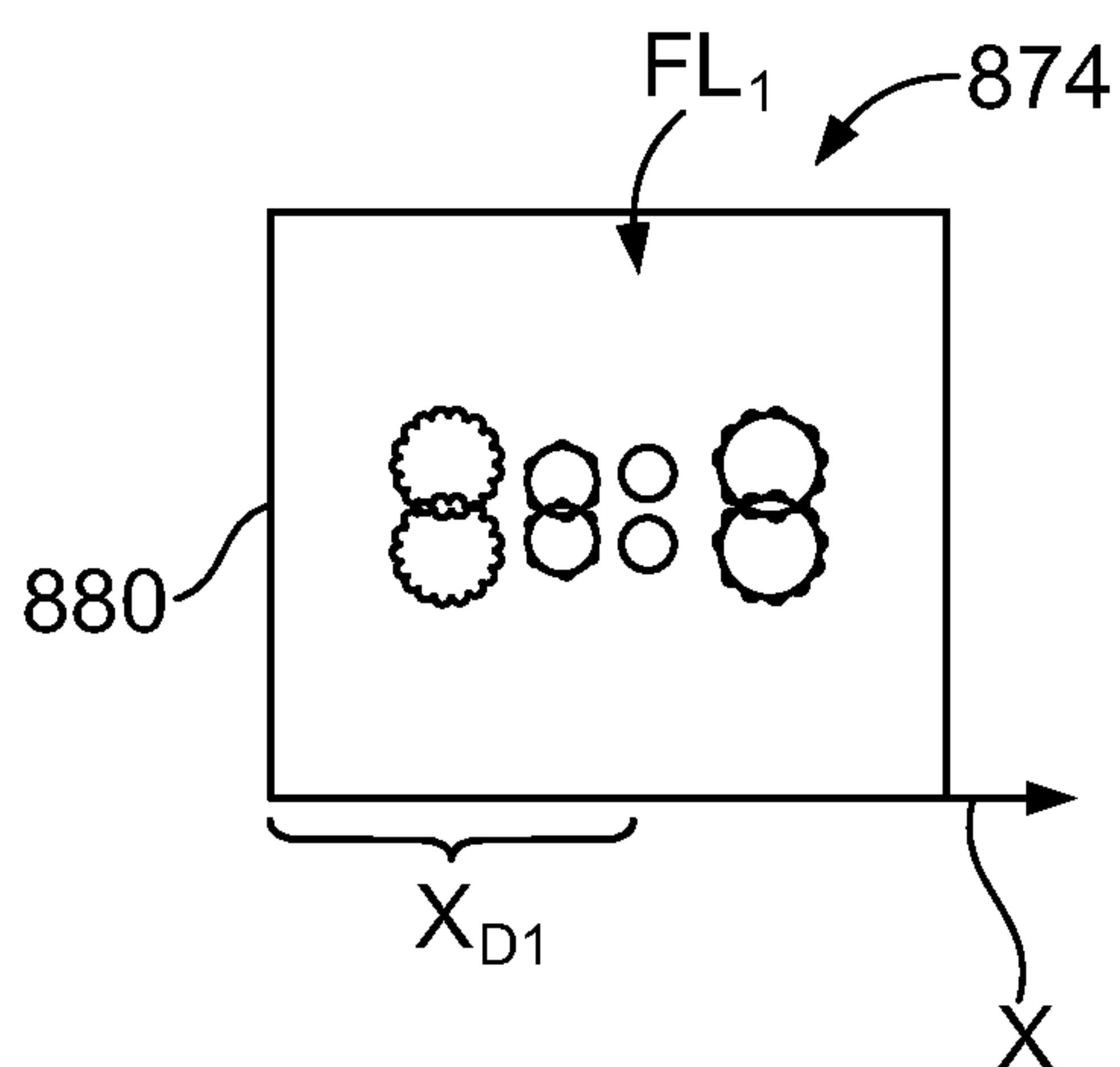


FIG. 57

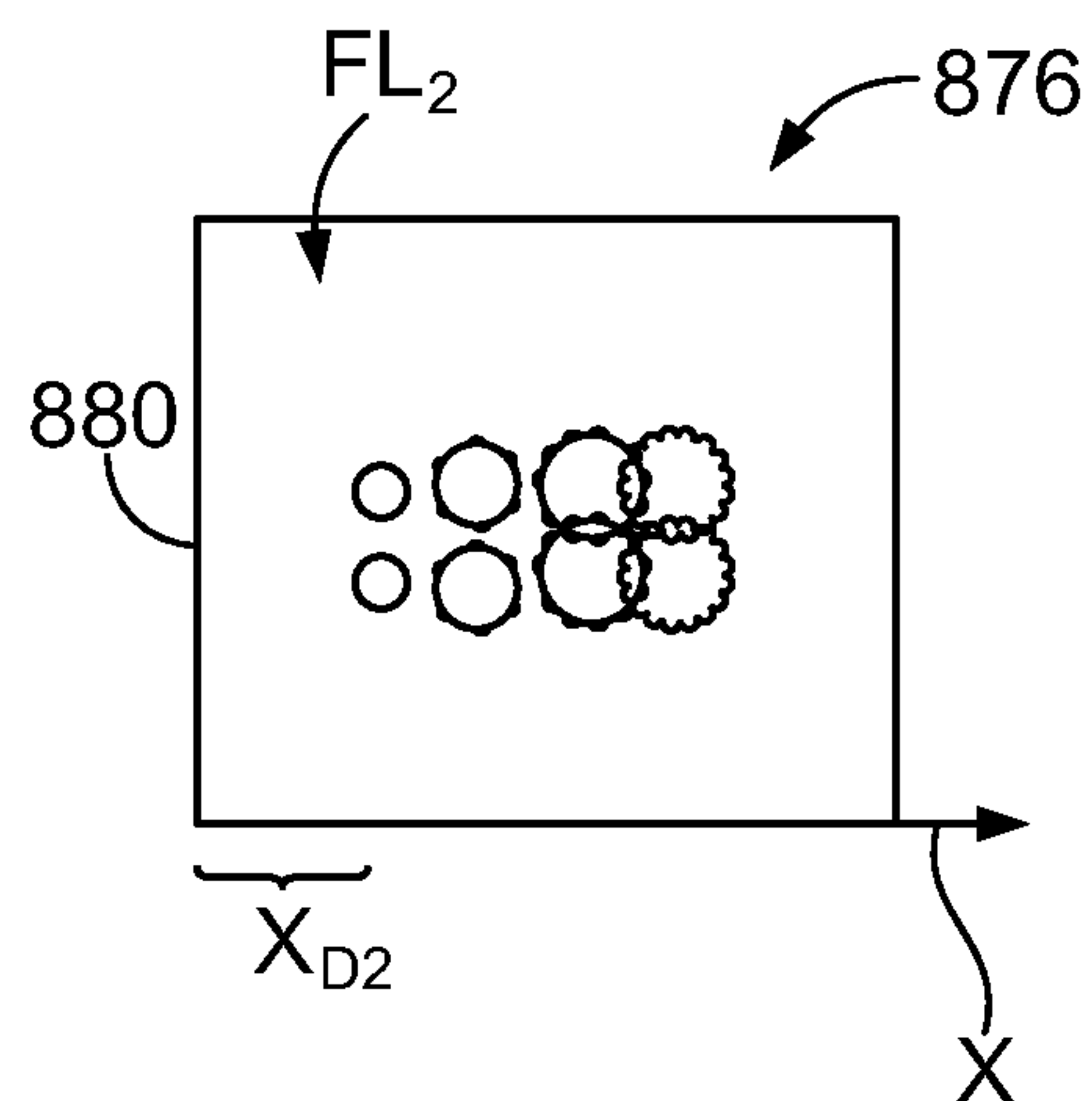


FIG. 58



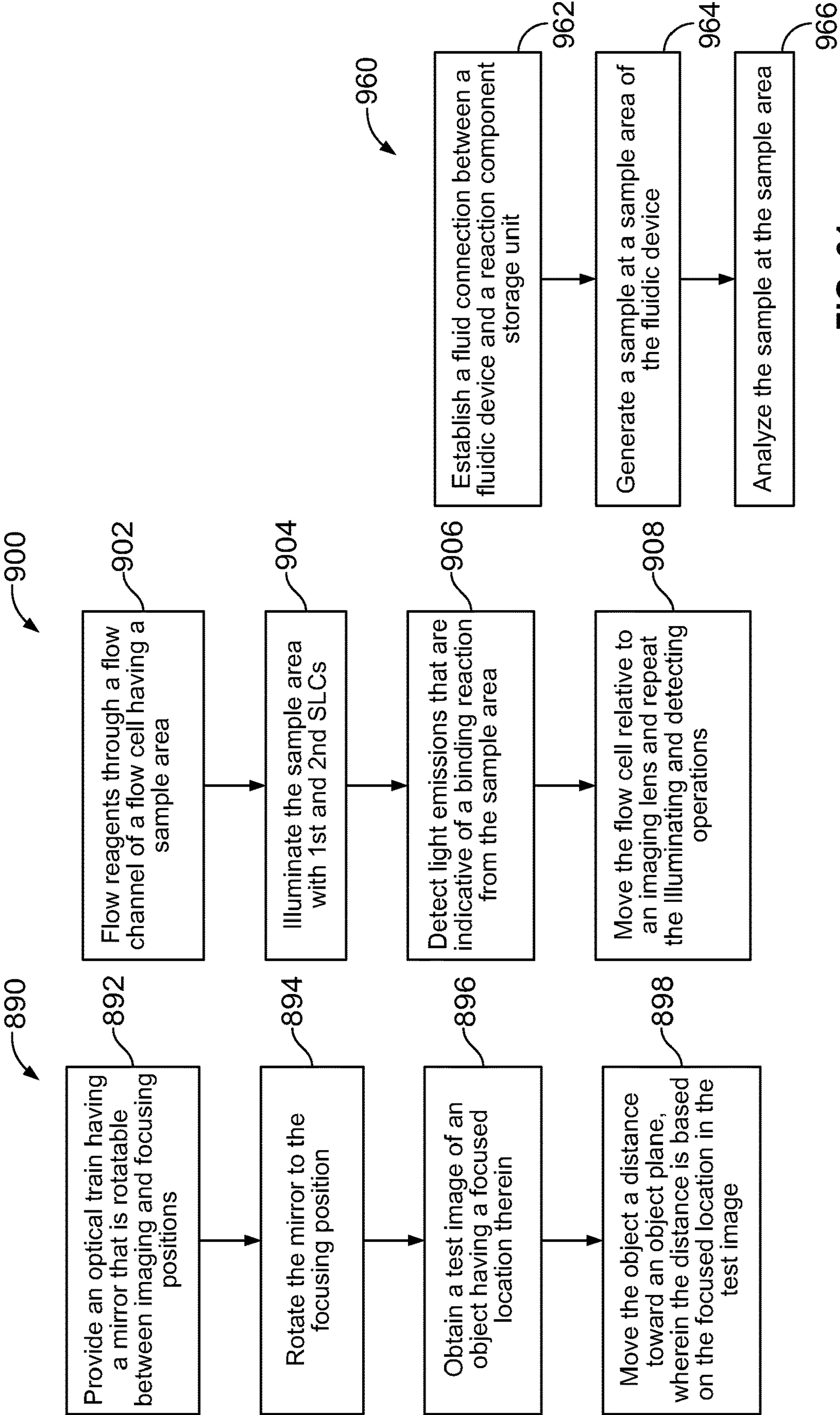


FIG. 59

FIG. 60

FIG. 61



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1

**SYSTEMS, METHODS, AND APPARATUSES  
TO IMAGE A SAMPLE FOR BIOLOGICAL  
OR CHEMICAL ANALYSIS****CROSS REFERENCE TO RELATED  
APPLICATIONS**

The present application is a continuation of U.S. patent application Ser. No. 16/255,546, filed Jan. 23, 2019, which is divisional of U.S. patent application Ser. No. 14/550,956 (now U.S. Pat. No. 10,220,386), filed Nov. 22, 2014, which is a continuation of U.S. patent application Ser. No. 13/273,666 (now U.S. Pat. No. 8,951,781), filed Oct. 14, 2011, which claims the benefit of and priority to U.S. Provisional Patent Application No. 61/438,486, filed Feb. 1, 2011, U.S. Provisional Patent Application No. 61/438,567, filed Feb. 1, 2011, U.S. Provisional Patent Application No. 61,438,530, filed Feb. 1, 2011, U.S. Provisional Patent Application No. 61/431,440, filed Feb. 11, 2011, U.S. Provisional Patent Application No. 61/431,439, filed Feb. 11, 2011, U.S. Provisional Patent Application No. 61/431,425, filed Feb. 10, 2011, and U.S. Provisional Patent Application No. 61/431,429, filed Feb. 10, 2011, the content of each of which is incorporated by reference herein in its entirety and for all purposes.

**BACKGROUND OF THE INVENTION**

Embodiments of the present invention relate generally to biological or chemical analysis and more particularly, to assay systems having fluidic devices, optical assemblies, and/or other apparatuses that may be used in detecting desired reactions in a sample.

Various assay protocols used for biological or chemical research are concerned with performing a large number of controlled reactions. In some cases, the controlled reactions are performed on support surfaces. The desired reactions may then be observed and analyzed to help identify properties or characteristics of the chemicals involved in the desired reaction. For example, in some protocols, a chemical moiety that includes an identifiable label (e.g., fluorescent label) may selectively bind to another chemical moiety under controlled conditions. These chemical reactions may be observed by exciting the labels with radiation and detecting light emissions from the labels. The light emissions may also be provided through other means, such as chemiluminescence.

Examples of such protocols include DNA sequencing. In one sequencing-by-synthesis (SBS) protocol, clusters of clonal amplicons are formed through bridge PCR on a surface of a flow channel. After generating the clusters of clonal amplicons, the amplicons may be “linearized” to make single stranded DNA (sstDNA). A series of reagents is flowed into the flow cell to complete a cycle of sequencing. Each sequencing cycle extends the sstDNA by a single nucleotide (e.g., A, T, G, C) having a unique fluorescent label. Each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in one cycle. After nucleotides are added to the sstDNAs clusters, an image in four channels is taken (i.e., one for each fluorescent label). After imaging, the fluorescent label and the terminator are chemically cleaved from the sstDNA and the growing DNA strand is ready for another cycle. Several cycles of reagent delivery and optical detection can be repeated to determine the sequences of the clonal amplicons.

However, systems configured to perform such protocols may have limited capabilities and may not be cost-effective.

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Thus, there is a general need for improved systems, methods, and apparatuses that are capable of performing or being used during assay protocols, such as the SBS protocol described above, in a cost-effective, simpler, or otherwise improved manner.

**BRIEF DESCRIPTION OF THE INVENTION**

In accordance with one embodiment, a fluidic device for analyzing samples is provided. The fluidic device includes a flow cell having inlet and outlet ports and a flow channel extending therebetween. The flow cell is configured to hold a sample-of-interest. The fluidic device also includes a housing having a reception space that is configured to receive the flow cell. The reception space is sized and shaped to permit the flow cell to float relative to the housing. The fluidic device also includes a gasket that is coupled to the housing. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively.

In another embodiment, a removable cartridge configured to hold and facilitate positioning a flow cell for imaging is provided. The cartridge includes a removable housing that has a reception space configured to hold the flow cell substantially within an object plane. The housing includes a pair of housing sides that face in opposite directions. The reception space extends along at least one of the housing sides so that the flow cell is exposed to an exterior of the housing through said at least one of the housing sides. The cartridge also includes a cover member that is coupled to the housing and includes a gasket. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is configured to be mounted over an exposed portion of the flow cell when the flow cell is held by the housing.

In yet another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes positioning a removable fluidic device on a support surface of an imaging system. The device has a reception space, a flow cell located within the reception space, and a gasket. The flow cell extends along an object plane in the reception space and is floatable relative to the gasket within the object plane. The method also includes moving the flow cell within the reception space while on the support surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket.

In another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes providing a fluidic device having a housing that includes a reception space and a floatable flow cell located within the reception space. The housing has recesses that are located immediately adjacent to the reception space. The method also includes positioning the fluidic device on a support structure having alignment members. The alignment members are inserted through corresponding recesses. The method also includes moving the flow cell within the reception space. The alignment members engage edges of the flow cell when the flow cell is moved within the reception space.

In another embodiment, a fluidic device holder is provided that is configured to orient a sample area with respect to mutually perpendicular X, Y, and Z-axes. The device holder includes a support structure that is configured to receive a fluidic device. The support structure includes a base surface that faces in a direction along the Z-axis and is configured to have the device positioned thereon. The device



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holder also includes a plurality of reference surfaces in respective directions along an XY-plane and an alignment assembly that includes an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end toward and away from the reference surfaces. The locator arm is configured to hold the device against the reference surfaces when the locator arm is in the biased position.

In another embodiment, a fluidic device holder is provided that includes a support structure having a loading region for receiving a fluidic device. The support structure includes a base surface that partially defines the loading region and is configured to have the device positioned thereon. The device holder includes a cover assembly that is coupled to the support structure and is configured to be removably mounted over the device. The cover assembly includes a cover housing having housing legs and a bridge portion that joins the housing legs. The housing legs extend in a common direction and have a viewing space that is located therebetween. The viewing space is positioned above the loading region.

In another embodiment, a method for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes is provided. The method includes providing an alignment assembly that has a movable locator arm having an engagement end. The locator arm is movable between retracted and biased positions. The method also includes positioning a fluidic device on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. The device has a sample area. The method also includes moving the locator arm to the biased position. The locator arm presses the device against the reference surfaces such that the device is held in a fixed position.

In yet another embodiment, an optical assembly is provided that includes a base plate having a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The optical assembly also includes an optical component having an optical surface that is configured to reflect light or transmit light therethrough. The optical assembly also includes a mounting device that has a component retainer and a biasing element that is operatively coupled to the retainer. The retainer holds the optical component so that a space portion of the optical surface faces the reference surface and a path portion of the optical surface extends beyond the support side into an optical path. The biasing element provides an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, a method of assembling an optical train is provided. The method includes providing a base plate that has a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The method also includes inserting an optical component into the component-receiving space. The optical component has an optical surface that is configured to reflect light or transmit light therethrough. The optical surface has a space portion that faces the reference surface and a path portion that

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extends beyond the support side into an optical path. The method also includes providing an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, an optical imaging system is provided that includes an object holder to hold and move an object and a detector to detect optical signals from the object at a detector surface. The imaging system also includes an optical train that is configured to direct the optical signals onto the detector surface. The optical train has an object plane that is proximate to the object holder and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The imaging system also includes an image analysis module that is configured to analyze a test image detected at the detector surface when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location in the test image is indicative of a position of the object with respect to the object plane. The object holder is configured to move the object toward the object plane based on the focused location.

In another embodiment, a method for controlling focus of an optical imaging system is provided. The method includes providing an optical train that is configured to direct optical signals onto a detector surface. The optical train has an object plane that is proximate to an object and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The method also includes rotating the mirror to the focusing position and obtaining a test image of the object when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location is indicative of a position of the object with respect to the object plane. The method also includes moving the object toward the object plane based on the focused location.

In another embodiment, an optical imaging system is provided that includes a sample holder configured to hold a flow cell. The flow cell includes a flow channel having a sample area. The imaging system also includes a flow system that is coupled to the flow cell and configured to direct reagents through the flow channel to the sample area. The imaging system also includes an optical train that is configured to direct excitation light onto the sample area and first and second light sources. The first and second light sources have fixed positions with respect to the optical train. The first and second light sources provide first and second optical signals, respectively, for exciting the biomolecules. The imaging system also includes a system controller that is communicatively coupled to the first and second light sources and to the flow system. The controller is configured to activate the flow system to flow the reagents to the sample area and activate the first and second light sources after a predetermined synthesis time period. The light sources can be, for example, lasers or semiconductor light sources (SLSs), such as laser diodes or light emitting diodes (LEDs).

In another embodiment, a method of performing a biological assay is provided. The method includes flowing reagents through a flow channel having a sample area. The sample area includes biomolecules that are configured to chemically react with the reagents. The method also includes



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illuminating the sample area with first and second light sources. The first and second light sources provide first and second optical signals, respectively. The biomolecules provide light emissions indicative of a binding reaction when illuminated by the first or second light sources. The method also includes detecting the light emissions from the sample area. The light sources can be, for example, lasers or semiconductor light sources (SLSs), such as a laser diodes or light emitting diodes (LEDs).

In another embodiment, a flow cell is provided that includes a first layer that has a mounting surface and an outer surface that face in opposite directions and that define a thickness therebetween. The flow cell also includes a second layer having a channel surface and an outer surface that face in opposite directions and that define a thickness therebetween. The second layer has a grooved portion that extends along the channel surface. The channel surface of the second layer is secured to the mounting surface. The flow cell also includes a flow channel that is defined by the grooved portion of the channel surface and a planar section of the mounting surface. The flow channel includes an imaging portion. The thickness of the second layer is substantially uniform along the imaging portion and is configured to transmit optical signals therethrough. The thickness of the first layer is substantially uniform along the imaging portion and is configured to permit uniform transfer of thermal energy therethrough.

In another embodiment, a light source module is provided that includes a module frame having a light passage and a light source that is secured to the module frame and oriented to direct optical signals through the light passage along an optical path. The light source module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the light source. The optical component is located within the light passage such that the optical component is within the optical path.

In another embodiment, an excitation light module is provided that includes a module frame and first and second semiconductor light sources (SLSs) that are secured to the module frame. The first and second SLSs have fixed positions with respect to each other. The first and second SLSs are configured to provide different excitation optical signals. The excitation light module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the first and second SLSs. The optical component permits the optical signals from the first SLS to transmit therethrough and reflects the optical signals from the second SLS. The reflected and transmitted optical signals are directed along a common path out of the module frame.

In one embodiment, a method of performing a biological or chemical assay is provided. The method includes establishing a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays. The reaction components include sample-generation components and sample-analysis components. The method also includes generating a sample at the sample area of the fluidic device. The generating operation includes flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. The method also includes analyzing the sample at the sample area. The analyzing operation includes flowing at least one sample-analysis component to the sample area. Said at least one sample-analysis component reacts with the sample to pro-

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vide optically detectable signals indicative of an event-of-interest. The generating and analyzing operations are conducted in an automated manner by the assay system.

In another embodiment, an assay system is provided that includes a fluidic device holder that is configured to hold a fluidic device and establish a fluid connection with the fluidic device. The assay system also includes a fluidic network that is configured to fluidically connect the fluidic device to a reaction component storage unit. The assay system also includes a fluidic control system that is configured to selectively flow fluids from the storage unit through the fluidic device. Furthermore, the assay system includes a system controller that has a fluidic control module. The fluidic control module is configured to instruct the fluidic control system to (a) flow different sample-generation components from the storage unit to the sample area and control reaction conditions at the sample area to generate a sample; and (b) flow at least one sample-analysis component from the storage unit to the sample area. Said at least one sample-analysis component is configured to react with the sample to provide optically detectable signals indicative of an event-of-interest. The assay system also includes an imaging system that is configured to detect the optically detectable signals from the sample. The system controller is configured to automatically generate the sample and analyze the sample by selectively controlling the fluidic device holder, the fluidic control system, and the imaging system.

In another embodiment, a method of performing a biological or chemical assay is provided. The method includes: (a) providing a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays, the reaction components including sample-generation components and sample-analysis components; (b) flowing sample generation components according to a predetermined protocol to generate a sample at the sample area; (c) selectively controlling reaction conditions at the sample area to facilitate generating the sample; (d) flowing sample-analysis components to the sample area; and (e) detecting optical signals emitted from the sample area, the optical signals being indicative of an event-of-interest between the sample-analysis components and the sample; wherein (b)-(e) are conducted in an automated manner.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of an assay system for performing biological or chemical assays formed in accordance with one embodiment.

FIG. 2 is a side view of a workstation configured to perform biological or chemical assays in accordance with one embodiment.

FIG. 3 is a front view of the workstation of FIG. 2.

FIG. 4 is a diagram of a fluidic network formed in accordance with one embodiment.

FIG. 5 is a perspective view of a flow cell formed in accordance with one embodiment.

FIG. 6 is a cross-section of the flow cell shown in FIG. 5 taken along the line 6-6 in FIG. 5.

FIG. 7 is a plan view of the flow cell of FIG. 5.

FIG. 8 is an enlarged view of a curved segment of a flow channel.

FIG. 9 is a perspective view of a fluidic device formed in accordance with one embodiment.

FIG. 10 is another perspective view of the fluidic device of FIG. 9.



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FIG. 11 is a cross-section of the fluidic device of FIG. 9 taken along the lines 11-11 in FIG. 9.

FIG. 12 is a perspective view of a fluidic device formed in accordance with another embodiment.

FIG. 13 is a perspective view of the fluidic device of FIG. 12.

FIG. 14 is a plan view of a fluidic device formed in accordance with one embodiment.

FIG. 15 is a side perspective view of the fluidic device of FIG. 14.

FIG. 16 is a partially exploded view of a device holder formed in accordance with one embodiment.

FIG. 17 is a perspective view of the assembled holder of FIG. 16.

FIG. 18 is a perspective view of a support structure that may be used in the holder of FIG. 16.

FIG. 19 is a top plan view of the holder of FIG. 16.

FIG. 20 is a perspective view of the holder of FIG. 16 having a cover assembly in an open position.

FIG. 21 is an enlarged plan view of the holder of FIG. 16.

FIG. 22 is a perspective view of a cover assembly that may be used in the holder of FIG. 16.

FIG. 23 is a cross-section of the cover assembly taken along the line 23-23 shown in FIG. 22.

FIG. 24 is a perspective view of a flow system that may be used with the holder of FIG. 16.

FIG. 25 is a block diagram of a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 26 is a block diagram illustrating a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 27 is a block diagram illustrating a method for orienting a sample area in accordance with one embodiment.

FIG. 28 is a perspective view of a fluid storage system formed in accordance with one embodiment.

FIG. 29 is a side cross-section of the fluid storage system of FIG. 28.

FIG. 30 is a perspective view of a removal assembly that may be used with the fluid storage system of FIG. 28.

FIG. 31 is a perspective view of a reaction component tray formed in accordance with one embodiment.

FIG. 32 is a top plan view of the tray shown in FIG. 31.

FIG. 33 is a side view of the tray shown in FIG. 31.

FIG. 34 is a front view of the tray shown in FIG. 31.

FIG. 35 is a side cross-section of a component well that may be used with the tray of FIG. 31.

FIG. 36 is a bottom perspective view of the component well of FIG. 35.

FIG. 37 is a perspective view of a component well that may be used with the tray of FIG. 31.

FIG. 38 is a diagram of an optical imaging system in accordance with one embodiment.

FIG. 39 is a perspective view of a motion-control system in accordance with one embodiment.

FIG. 40 is a perspective view of components that may be used with the motion-control system of FIG. 39.

FIG. 41 is a perspective view of an optical base plate that may be used in the imaging system of FIG. 38.

FIG. 42 is a plan view of the base plate of FIG. 41.

FIG. 43 is a perspective view of an optical component formed in accordance with one embodiment that may be used in the imaging system of FIG. 38.

FIG. 44 is a cut-away perspective view of the optical component of FIG. 43.

FIG. 45 is a front view of the optical component of FIG. 43.

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FIG. 46 is a side view of the optical component of FIG. 43 during a mounting operation.

FIG. 47 is a block diagram illustrating a method of assembling an optical train in accordance with one embodiment.

FIG. 48 is a perspective view of a light source module formed in accordance with one embodiment.

FIG. 49 is a side view of the light source module of FIG. 48.

FIG. 50 is a plan view of the light source module of FIG. 48.

FIG. 51 is a plan view of an image-focusing system in accordance with one embodiment.

FIG. 52 is a perspective view of a rotatable mirror assembly that may be used in the image-focusing system of FIG. 51.

FIG. 53 is a schematic diagram of a rotatable mirror in an imaging position that may be used in the image-focusing system of FIG. 51.

FIGS. 54 and 55 illustrate sample images that may be obtained by the image-focusing system of FIG. 51.

FIG. 56 is a schematic diagram of the rotatable mirror of FIG. 53 in a focusing position.

FIGS. 57 and 58 illustrate test images that may be obtained by the image-focusing system of FIG. 51.

FIG. 59 is a block diagram illustrating a method for controlling focus of an optical imaging system.

FIG. 60 illustrates a method for performing an assay for biological or chemical analysis.

FIG. 61 illustrates a method for performing an assay for biological or chemical analysis.

#### DETAILED DESCRIPTION OF THE INVENTION

Embodiments described herein include various systems, methods, assemblies, and apparatuses used to detect desired reactions in a sample for biological or chemical analysis. In some embodiments, the desired reactions provide optical signals that are detected by an optical assembly. The optical signals may be light emissions from labels or may be transmission light that has been reflected or refracted by the sample. For example, embodiments may be used to perform or facilitate performing a sequencing protocol in which ssDNA is sequenced in a flow cell. In particular embodiments, the embodiments described herein can also perform an amplification protocol to generate a sample-of-interest for sequencing.

As used herein, a “desired reaction” includes a change in at least one of a chemical, electrical, physical, and optical property or quality of a substance that is in response to a stimulus. For example, the desired reaction may be a chemical transformation, chemical change, or chemical interaction. In particular embodiments, the desired reactions are detected by an imaging system. The imaging system may include an optical assembly that directs optical signals to a sensor (e.g., CCD or CMOS). However, in other embodiments, the imaging system may detect the optical signals directly. For example, a flow cell may be mounted onto a CMOS sensor. However, the desired reactions may also be a change in electrical properties. For example, the desired reaction may be a change in ion concentration within a solution.

Exemplary reactions include, but are not limited to, chemical reactions such as reduction, oxidation, addition, elimination, rearrangement, esterification, amidation, etherification, cyclization, or substitution; binding interactions in



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which a first chemical binds to a second chemical; dissociation reactions in which two or more chemicals detach from each other; fluorescence; luminescence; chemiluminescence; and biological reactions, such as nucleic acid replication, nucleic acid amplification, nucleic acid hybridization, nucleic acid ligation, phosphorylation, enzymatic catalysis, receptor binding, or ligand binding. The desired reaction can also be addition or elimination of a proton, for example, detectable as a change in pH of a surrounding solution or environment.

The stimulus can be at least one of physical, optical, electrical, magnetic, and chemical. For example, the stimulus may be an excitation light that excites fluorophores in a substance. The stimulus may also be a change in a surrounding environment, such as a change in concentration of certain biomolecules (e.g., enzymes or ions) in a solution. The stimulus may also be an electrical current applied to a solution within a predefined volume. In addition, the stimulus may be provided by shaking, vibrating, or moving a reaction chamber where the substance is located to create a force (e.g., centripetal force). As used herein, the phrase “in response to a stimulus” is intended to be interpreted broadly and include more direct responses to a stimulus (e.g., when a fluorophore emits energy of a specific wavelength after absorbing incident excitation light) and more indirect responses to a stimulus in that the stimulus initiates a chain of events that eventually results in the response (e.g., incorporation of a base in pyrosequencing eventually resulting in chemiluminescence). The stimulus may be immediate (e.g., excitation light incident upon a fluorophore) or gradual (e.g., change in temperature of the surrounding environment).

As used herein, the phrase “activity that is indicative of a desired reaction” and variants thereof include any detectable event, property, quality, or characteristic that may be used to facilitate determining whether a desired reaction has occurred. The detected activity may be a light signal generated in fluorescence or chemiluminescence. The detected activity may also be a change in electrical properties of a solution within a predefined volume or along a predefined area. The detected activity may be a change in temperature.

Various embodiments include providing a reaction component to a sample. As used herein, a “reaction component” or “reactant” includes any substance that may be used to obtain a desired reaction. For example, reaction components include reagents, enzymes, samples, other biomolecules, and buffer solutions. The reaction components are typically delivered to a reaction site (e.g., area where sample is located) in a solution or immobilized within a reaction site. The reaction components may interact directly or indirectly with the substance of interest.

In particular embodiments, the desired reactions are detected optically through an optical assembly. The optical assembly may include an optical train of optical components that cooperate with one another to direct the optical signals to an imaging device (e.g., CCD, CMOS, or photomultiplier tubes). However, in alternative embodiments, the sample region may be positioned immediately adjacent to an activity detector that detects the desired reactions without the use of an optical train. The activity detector may be able to detect predetermined events, properties, qualities, or characteristics within a predefined volume or area. For example, an activity detector may be able to capture an image of the predefined volume or area. An activity detector may be able to detect an ion concentration within a predefined volume of a solution or along a predefined area. Exemplary activity detectors include charged-coupled devices (CCD’s) (e.g., CCD cameras); photomultiplier tubes (PMT’s); molecular

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characterization devices or detectors, such as those used with nanopores; microcircuit arrangements, such as those described in U.S. Pat. No. 7,595,883, which is incorporated herein by reference in the entirety; and CMOS-fabricated sensors having field effect transistors (FET’s), including chemically sensitive field effect transistors (chemFET), ion-sensitive field effect transistors (ISFET), and/or metal oxide semiconductor field effect transistors (MOSFET).

As used herein, the term “optical components” includes various elements that affect the propagation of optical signals. For example, the optical components may at least one of redirect, filter, shape, magnify, or concentrate the optical signals. The optical signals that may be affected include the optical signals that are upstream from the sample and the optical signals that are downstream from the sample. In a fluorescence-detection system, upstream components include those that direct excitation radiation toward the sample and downstream components include those that direct emission radiation away from the sample. Optical components may be, for example, reflectors, dichroics, beam splitters, collimators, lenses, filters, wedges, prisms, mirrors, detectors, and the like. Optical components also include bandpass filters, optical wedges, and optical devices similar to those described herein.

As used herein, the term “optical signals” or “light signals” includes electromagnetic energy capable of being detected. The term includes light emissions from labeled biological or chemical substances and also includes transmitted light that is refracted or reflected by optical substrates. Optical or light signals, including excitation radiation that is incident upon the sample and light emissions that are provided by the sample, may have one or more spectral patterns. For example, more than one type of label may be excited in an imaging session. In such cases, the different types of labels may be excited by a common excitation light source or may be excited by different excitation light sources at different times or at the same time. Each type of label may emit optical signals having a spectral pattern that is different from the spectral pattern of other labels. For example, the spectral patterns may have different emission spectra. The light emissions may be filtered to separately detect the optical signals from other emission spectra.

As used herein, when the term “different” is used with respect to light emissions (including emission spectra or other emission characteristics), the term may be interpreted broadly to include the light emissions being distinguishable or differentiable. For example, the emission spectra of the light emissions may have wavelength ranges that at least partially overlap so long as at least a portion of one emission spectrum does not completely overlap the other emission spectrum. Different emission spectra may also have the same or similar wavelength ranges, but have different intensities that are differentiable. Different optical signals can be distinguished based on different characteristics of excitation light that produces the optical signals. For example, in fluorescence resonance energy transfer (FRET) imaging, the light emissions may be the same but the cause (e.g., excitation optical signals) of the light emissions may be different. More specifically, a first excitation wavelength can be used to excite a donor fluorophore of a donor-acceptor pair such that FRET results in emission from the acceptor and excitation of the acceptor directly will also result in emission from the acceptor. As such, differentiation of the optical signals can be based on observation of an emission signal in combination with identification of the excitation wavelength used to produce the emission. Different light emissions may have other characteristics that do not overlap, such as



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emission anisotropy or fluorescence lifetime. Also, when the light emissions are filtered, the wavelength ranges of the emission spectra may be narrowed.

The optical components may have fixed positions in the optical assembly or may be selectively moveable. As used herein, when the term “selectively” is used in conjunction with “moving” and similar terms, the phrase means that the position of the optical component may be changed in a desired manner. At least one of the locations and the orientation of the optical component may be changed. For example, in particular embodiments, a rotatable mirror is selectively moved to facilitate focusing an optical imaging system.

Different elements and components described herein may be removably coupled. As used herein, when two or more elements or components are “removably coupled” (or “removably mounted,” and other like terms) the elements are readily separable without destroying the coupled components. For instance, elements can be readily separable when the elements may be separated from each other without undue effort, without the use of a tool (i.e. by hand), or without a significant amount of time spent in separating the components. By way of example, in some embodiments, an optical device may be removably mounted to an optical base plate. In addition, flow cells and fluidic devices may be removably mounted to a device holder.

Imaging sessions include a time period in which at least a portion of the sample is imaged. One sample may undergo or be subject to multiple imaging sessions. For example, one sample may be subject to two different imaging sessions in which each imaging session attempts to detect optical signals from one or more different labels. As a specific example, a first scan along at least a portion of a nucleic acid sample may detect labels associated with nucleotides A and C and a second scan along at least a portion of the sample may detect labels associated with nucleotides G and T. In sequencing embodiments, separate sessions can occur in separate cycles of a sequencing protocol. Each cycle can include one or more imaging session. In other embodiments, detecting optical signals in different imaging sessions may include scanning different samples. Different samples may be of the same type (e.g., two microarray chips) or of different types (e.g., a flow cell and a microarray chip).

During an imaging session, optical signals provided by the sample are observed. Various types of imaging may be used with embodiments described herein. For example, embodiments described herein may utilize a “step and shoot” procedure in which regions of a sample area are individually imaged. Embodiments may also be configured to perform at least one of epi-fluorescent imaging and total-internal-reflectance-fluorescence (TIRF) imaging. In other embodiments, the sample imager is a scanning time-delay integration (TDI) system. Furthermore, the imaging sessions may include “line scanning” one or more samples such that a linear focal region of light is scanned across the sample(s). Some methods of line scanning are described, for example, in U.S. Pat. No. 7,329,860 and U.S. Pat. Pub. No. 2009/0272914, each of which the complete subject matter is incorporated herein by reference in their entirety. Imaging sessions may also include moving a point focal region of light in a raster pattern across the sample(s). In alternative embodiments, imaging sessions may include detecting light emissions that are generated, without illumination, and based entirely on emission properties of a label within the sample (e.g., a radioactive or chemiluminescent component

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in the sample). In alternative embodiments, flow cells may be mounted onto an imager (e.g., CCD or CMOS) that detects the desired reactions.

As used herein, the term “sample” or “sample-of-interest” includes various materials or substances of interest that undergo an imaging session where optical signals from the material or substance are observed. In particular embodiments, a sample may include biological or chemical substances of interests and, optionally, an optical substrate or support structure that supports the biological or chemical substances. As such, a sample may or may not include an optical substrate or support structure. As used herein, the term “biological or chemical substances” may include a variety of biological or chemical substances that are suitable for being imaged or examined with the optical systems described herein. For example, biological or chemical substances include biomolecules, such as nucleosides, nucleic acids, polynucleotides, oligonucleotides, proteins, enzymes, polypeptides, antibodies, antigens, ligands, receptors, polysaccharides, carbohydrates, polyphosphates, nanopores, organelles, lipid layers, cells, tissues, organisms, and biologically active chemical compound(s) such as analogs or mimetics of the aforementioned species. Other chemical substances include labels that can be used for identification, examples of which include fluorescent labels and others set forth in further detail below.

Different types of samples may include different optical substrates or support structures that affect incident light in different manners. In particular embodiments, samples to be detected can be attached to one or more surfaces of a substrate or support structure. For example, flow cells may include one or more flow channels. In flow cells, the flow channels may be separated from the surrounding environment by top and bottom layers of the flow cell. Thus, optical signals to be detected are projected from within the support structure and may transmit through multiple layers of material having different refractive indices. For example, when detecting optical signals from an inner bottom surface of a flow channel and when detecting optical signals from above the flow channel, the optical signals that are desired to be detected may propagate through a fluid having an index of refraction, through one or more layers of the flow cells having different indices of refraction, and through the ambient environment having a different index of refraction.

As used herein, a “fluidic device” is an apparatus that includes one or more flow channels that direct fluid in a predetermined manner to conduct desired reactions. The fluidic device is configured to be fluidically coupled to a fluidic network of an assay system. By way of example, a fluidic device may include flow cells or lab-on-chip devices. Flow cells generally hold a sample along a surface for imaging by an external imaging system. Lab-on-chip devices may hold the sample and perform additional functions, such as detecting the desired reaction using an integrated detector. Fluidic devices may optionally include additional components, such as housings or imagers, that are operatively coupled to the flow channels. In particular embodiments, the channels may have channel surfaces where a sample is located, and the fluidic device can include a transparent material that permits the sample to be imaged after a desired reaction occurs.

In particular embodiments, the fluidic devices have channels with microfluidic dimensions. In such channels, the surface tension and cohesive forces of the liquid flowing therethrough and the adhesive forces between the liquid and the surfaces of the channel have at least a substantial effect on the flow of the liquid. For example, a cross-sectional area



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(taken perpendicular to a flow direction) of a microfluidic channel may be about  $10\text{ }\mu\text{m}^2$  or less.

In alternative embodiments, optical imaging systems described herein may be used to scan samples that include microarrays. A microarray may include a population of different probe molecules that are attached to one or more substrates such that the different probe molecules can be differentiated from each other according to relative location. An array can include different probe molecules, or populations of the probe molecules, that are each located at a different addressable location on a substrate. Alternatively, a microarray can include separate optical substrates, such as beads, each bearing a different probe molecule, or population of the probe molecules, that can be identified according to the locations of the optical substrates on a surface to which the substrates are attached or according to the locations of the substrates in a liquid. Exemplary arrays in which separate substrates are located on a surface include, without limitation, a BeadChip Array available from Illumina®, Inc. (San Diego, Calif.) or others including beads in wells such as those described in U.S. Pat. Nos. 6,266,459, 6,355,431, 6,770,441, 6,859,570, and 7,622,294; and PCT Publication No. WO 00/63437, each of which is hereby incorporated by reference. Other arrays having particles on a surface include those set forth in US 2005/0227252; WO 05/033681; and WO 04/024328, each of which is hereby incorporated by reference.

Any of a variety of microarrays known in the art can be used. A typical microarray contains sites, sometimes referred to as features, each having a population of probes. The population of probes at each site is typically homogenous having a single species of probe, but in some embodiments the populations can each be heterogeneous. Sites or features of an array are typically discrete, being separated. The separate sites can be contiguous or they can have spaces between each other. The size of the probe sites and/or spacing between the sites can vary such that arrays can be high density, medium density or lower density. High density arrays are characterized as having sites separated by less than about  $15\text{ }\mu\text{m}$ . Medium density arrays have sites separated by about  $15$  to  $30\text{ }\mu\text{m}$ , while low density arrays have sites separated by greater than  $30\text{ }\mu\text{m}$ . An array useful in the invention can have sites that are separated by less than  $100\text{ }\mu\text{m}$ ,  $50\text{ }\mu\text{m}$ ,  $10\text{ }\mu\text{m}$ ,  $5\text{ }\mu\text{m}$ ,  $1\text{ }\mu\text{m}$ , or  $0.5\text{ }\mu\text{m}$ . An apparatus or method of an embodiment of the invention can be used to image an array at a resolution sufficient to distinguish sites at the above densities or density ranges.

Further examples of commercially available microarrays that can be used include, for example, an Affymetrix® GeneChip® microarray or other microarray synthesized in accordance with techniques sometimes referred to as VLSIPS™ (Very Large Scale Immobilized Polymer Synthesis) technologies as described, for example, in U.S. Pat. Nos. 5,324,633; 5,744,305; 5,451,683; 5,482,867; 5,491,074; 5,624,711; 5,795,716; 5,831,070; 5,856,101; 5,858,659; 5,874,219; 5,968,740; 5,974,164; 5,981,185; 5,981,956; 6,025,601; 6,033,860; 6,090,555; 6,136,269; 6,022,963; 6,083,697; 6,291,183; 6,309,831; 6,416,949; 6,428,752 and 6,482,591, each of which is hereby incorporated by reference. A spotted microarray can also be used in a method according to an embodiment of the invention. An exemplary spotted microarray is a CodeLink™ Array available from Amersham Biosciences. Another microarray that is useful is one that is manufactured using inkjet printing methods such as SurePrint™ Technology available from Agilent Technologies.

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The systems and methods set forth herein can be used to detect the presence of a particular target molecule in a sample contacted with the microarray. This can be determined, for example, based on binding of a labeled target analyte to a particular probe of the microarray or due to a target-dependent modification of a particular probe to incorporate, remove, or alter a label at the probe location. Any one of several assays can be used to identify or characterize targets using a microarray as described, for example, in U.S. Patent Application Publication Nos. 2003/0108867; 2003/0108900; 2003/0170684; 2003/0207295; or 2005/0181394, each of which is hereby incorporated by reference.

Furthermore, optical systems described herein may be constructed to include various components and assemblies as described in PCT application PCT/US07/07991, entitled “System and Devices for Sequence by Synthesis Analysis”, filed Mar. 30, 2007 and/or to include various components and assemblies as described in International Publication No. WO 2009/042862, entitled “Fluorescence Excitation and Detection System and Method”, filed Sep. 26, 2008, both of which the complete subject matter are incorporated herein by reference in their entirety. In particular embodiments, optical systems can include various components and assemblies as described in U.S. Pat. No. 7,329,860 and WO 2009/137435, of which the complete subject matter is incorporated herein by reference in their entirety. Optical systems can also include various components and assemblies as described in U.S. patent application Ser. No. 12/638,770, filed on Dec. 15, 2009, of which the complete subject matter is incorporated herein by reference in its entirety.

In particular embodiments, methods, and optical systems described herein may be used for sequencing nucleic acids. For example, sequencing-by-synthesis (SBS) protocols are particularly applicable. In SBS, a plurality of fluorescently labeled modified nucleotides are used to sequence a plurality of clusters of amplified DNA (possibly millions of clusters) present on the surface of an optical substrate (e.g., a surface that at least partially defines a channel in a flow cell). The flow cells may contain nucleic acid samples for sequencing where the flow cells are placed within the appropriate flow cell holders. The samples for sequencing can take the form of single nucleic acid molecules that are separated from each other so as to be individually resolvable, amplified populations of nucleic acid molecules in the form of clusters or other features, or beads that are attached to one or more molecules of nucleic acid. Accordingly, sequencing can be carried out on an array such as those set forth previously herein. The nucleic acids can be prepared such that they comprise an oligonucleotide primer adjacent to an unknown target sequence. To initiate the first SBS sequencing cycle, one or more differently labeled nucleotides, and DNA polymerase, etc., can be flowed into/through the flow cell by a fluid flow subsystem (not shown). Either a single type of nucleotide can be added at a time, or the nucleotides used in the sequencing procedure can be specially designed to possess a reversible termination property, thus allowing each cycle of the sequencing reaction to occur simultaneously in the presence of several types of labeled nucleotides (e.g. A, C, T, G). The nucleotides can include detectable label moieties such as fluorophores. Where the four nucleotides are mixed together, the polymerase is able to select the correct base to incorporate and each sequence is extended by a single base. Nonincorporated nucleotides can be washed away by flowing a wash solution through the flow cell. One or more lasers may excite the nucleic acids and induce fluorescence. The fluorescence emitted from the nucleic acids is based upon the fluorophores of the incorporated



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base, and different fluorophores may emit different wavelengths of emission light. A deblocking reagent can be added to the flow cell to remove reversible terminator groups from the DNA strands that were extended and detected. The deblocking reagent can then be washed away by flowing a wash solution through the flow cell. The flow cell is then ready for a further cycle of sequencing starting with introduction of a labeled nucleotide as set forth above. The fluidic and detection steps can be repeated several times to complete a sequencing run. Exemplary sequencing methods are described, for example, in Bentley et al., *Nature* 456:53-59 (2008), WO 04/018497; U.S. Pat. No. 7,057,026; WO 91/06678; WO 07/123744; U.S. Pat. Nos. 7,329,492; 7,211,414; 7,315,019; 7,405,281, and US 2008/0108082, each of which is incorporated herein by reference.

In some embodiments, nucleic acids can be attached to a surface and amplified prior to or during sequencing. For example, amplification can be carried out using bridge amplification to form nucleic acid clusters on a surface. Useful bridge amplification methods are described, for example, in U.S. Pat. No. 5,641,658; U.S. Patent Publ. No. 2002/0055100; U.S. Pat. No. 7,115,400; U.S. Patent Publ. No. 2004/0096853; U.S. Patent Publ. No. 2004/0002090; U.S. Patent Publ. No. 2007/0128624; and U.S. Patent Publ. No. 2008/0009420. Another useful method for amplifying nucleic acids on a surface is rolling circle amplification (RCA), for example, as described in Lizardi et al., *Nat. Genet.* 19:225-232 (1998) and US 2007/0099208 A1, each of which is incorporated herein by reference. Emulsion PCR on beads can also be used, for example as described in Dressman et al., *Proc. Natl. Acad. Sci. USA* 100:8817-8822 (2003), WO 05/010145, or U.S. Patent Publ. Nos. 2005/0130173 or 2005/0064460, each of which is incorporated herein by reference in its entirety.

Other sequencing techniques that are applicable for use of the methods and systems set forth herein are pyrosequencing, nanopore sequencing, and sequencing by ligation. Exemplary pyrosequencing techniques and samples that are particularly useful are described in U.S. Pat. Nos. 6,210,891; 6,258,568; 6,274,320 and Ronaghi, *Genome Research* 11:3-11 (2001), each of which is incorporated herein by reference. Exemplary nanopore techniques and samples that are also useful are described in Deamer et al., *Acc. Chem. Res.* 35:817-825 (2002); Li et al., *Nat. Mater.* 2:611-615 (2003); Soni et al., *Clin Chem.* 53:1996-2001 (2007) Healy et al., *Nanomed.* 2:459-481 (2007) and Cockroft et al., *J. am. Chem. Soc.* 130:818-820; and U.S. Pat. No. 7,001,792, each of which is incorporated herein by reference. In particular, these methods utilize repeated steps of reagent delivery. An instrument or method set forth herein can be configured with reservoirs, valves, fluidic lines and other fluidic components along with control systems for those components in order to introduce reagents and detect optical signals according to a desired protocol such as those set forth in the references cited above. Any of a variety of samples can be used in these systems such as substrates having beads generated by emulsion PCR, substrates having zero-mode waveguides, substrates having integrated CMOS detectors, substrates having biological nanopores in lipid bilayers, solid-state substrates having synthetic nanopores, and others known in the art. Such samples are described in the context of various sequencing techniques in the references cited above and further in US 2005/0042648; US 2005/0079510; US 2005/0130173; and WO 05/010145, each of which is incorporated herein by reference.

Exemplary labels that can be detected in accordance with various embodiments, for example, when present on or

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within a support structure include, but are not limited to, a chromophore; luminophore; fluorophore; optically encoded nanoparticles; particles encoded with a diffraction-grating; electrochemiluminescent label such as Ru(bpy)<sup>32+</sup>; or moiety that can be detected based on an optical characteristic. Fluorophores that may be useful include, for example, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malachite green, Cy3, Cy5, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, alexa dyes, phycoerythrin, bodipy, and others known in the art such as those described in Haugland, *Molecular Probes Handbook*, (Eugene, Oreg.) 6th Edition; The Synthesgen catalog (Houston, Tex.), Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Ed., Plenum Press New York (1999), or WO 98/59066, each of which is hereby incorporated by reference. In some embodiments, the one pair of labels may be excitable by a first excitation wavelength and another pair of labels may be excitable by a second excitation wavelength.

Although embodiments are exemplified with regard to detection of samples that include biological or chemical substances supported by an optical substrate, it will be understood that other samples can be imaged by the embodiments described herein. Other exemplary samples include, but are not limited to, biological specimens such as cells or tissues, electronic chips such as those used in computer processors, and the like. Examples of some of the applications include microscopy, satellite scanners, high-resolution reprographics, fluorescent image acquisition, analyzing and sequencing of nucleic acids, DNA sequencing, sequencing-by-synthesis, imaging of microarrays, imaging of holographically encoded microparticles and the like.

FIG. 1 is a block diagram of an assay system 100 for biological or chemical analysis formed in accordance with one embodiment. In some embodiments, the assay system 100 is a workstation that may be similar to a bench-top device or desktop computer. For example, at least a majority of the systems and components for conducting the desired reactions can be within a common housing 117 of the assay system 100. In other embodiments, the assay system 100 includes one or more components, assemblies, or systems that are remotely located from the assay system 100 (e.g., a remote database). The assay system 100 may include various components, assemblies, and systems (or sub-systems) that interact with each other to perform one or more predetermined methods or assay protocols for biological or chemical analysis.

For example, the assay system 100 includes a system controller 102 that may communicate with the various components, assemblies, and systems (or sub-systems) of the assay system 100. As shown, the assay system 100 has an optical assembly 104, an excitation source assembly 106, a detector assembly 108, and a fluidic device holder 110 that supports one or more fluidic devices 112 having a sample thereon. The fluidic device may be a flow cell, such as the flow cell 200 described below, or the fluidic device 112 may be the fluidic device 300 described below.

In some embodiments, the optical assembly 104 is configured to direct incident light from the excitation source assembly 106 onto the fluidic device(s) 112. The excitation source assembly 106 may include one or more excitation light sources that are configured to excite labels associated with the sample. The excitation source assembly 106 may also be configured to provide incident light that is reflected and/or refracted by the samples. As shown, the samples may provide optical signals that include light emissions 116



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and/or transmission light **118**. The device holder **110** and the optical assembly **104** may be moved relative to each other. In some embodiments, the device holder **110** includes a motor assembly **132** that moves the fluidic device **112** with respect to the optical assembly **104**. In other embodiments, the optical assembly **104** may be moved in addition to or alternatively to the device holder **110**. The optical assembly **104** may also be configured to direct the light emissions **116** and/or transmission light **118** to the detector assembly **108**. The detector assembly **108** may include one or more imaging detectors. The imaging detectors may be, by way of example only, CCD or CMOS cameras, or photomultiplier tubes.

Also shown, the assay system **100** may include a fluidic control system **134** to control the flow of fluid throughout a fluidic network **135** (indicated by the solid lines) of the assay system **100**. The fluidic control system **134** may deliver reaction components (e.g., reagents) or other fluids to the fluidic device **112** during, for example, a sequencing protocol. The assay system **100** may also include a fluid storage system **136** that is configured to hold fluids that may be used by the assay system **100** and a temperature control system **138** that regulates the temperature of the fluid. The temperature control system **138** may also generally regulate a temperature of the assay system **100** using, for example, thermal modules, heat sinks, and blowers.

Also shown, the assay system **100** may include a user interface **140** that interacts with the user. For example, the user interface **140** may include a display **142** to display or request information from a user and a user input device **144** to receive user inputs. In some embodiments, the display **142** and the user input device **144** are the same device (e.g., touchscreen). As will be discussed in greater detail below, the assay system **100** may communicate with various components to perform the desired reactions. The assay system **100** may also be configured to analyze the detection data to provide a user with desired information.

The fluidic control system **134** is configured to direct and regulate the flow of one or more fluids through the fluidic network **135**. The fluidic control system **134** may include, for example, pumps and valves that are selectively operable for controlling fluid flow. The fluidic network **135** may be in fluid communication with the fluidic device **112** and the fluid storage system **136**. For example, select fluids may be drawn from the fluid storage system **136** and directed to the fluidic device **112** in a controlled manner, or the fluids may be drawn from the fluidic device **112** and directed toward, for example, a waste reservoir in the fluid storage system **136**. Although not shown, the fluidic control system **134** may also include flow sensors that detect a flow rate or pressure of the fluids within the fluidic network. The sensors may communicate with the system controller **102**.

The temperature control system **138** is configured to regulate the temperature of fluids at different regions of the fluidic network **135**, the fluid storage system **136**, and/or the fluidic device **112**. For example, the temperature control system **138** may include a thermocycler **113** that interfaces with the fluidic device **112** and controls the temperature of the fluid that flows along the fluidic device **112**. Although not shown, the temperature control system **138** may include sensors to detect the temperature of the fluid or other components. The sensors may communicate with the system controller **102**.

The fluid storage system **136** is in fluid communication with the fluidic device **112** and may store various reaction components or reactants that are used to conduct the desired reactions therein. The fluid storage system **136** may store

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fluids for washing or cleaning the fluidic network **135** or the fluidic device **112** and also for diluting the reactants. For example, the fluid storage system **136** may include various reservoirs to store reagents, enzymes, other biomolecules, buffer solutions, aqueous, and non-polar solutions, and the like. Furthermore, the fluid storage system **136** may also include waste reservoirs for receiving waste products.

The device holder **110** is configured to engage one or more fluidic devices **112**, for example, in at least one of a mechanical, electrical, and fluidic manner. The device holder **110** may hold the fluidic device(s) **112** in a desired orientation to facilitate the flow of fluid through the fluidic device **112** and/or imaging of the fluidic device **112**.

The system controller **102** may include any processor-based or microprocessor-based system, including systems using microcontrollers, reduced instruction set computers (RISC), application specific integrated circuits (ASICs), field programmable gate array (FPGAs), logic circuits, and any other circuit or processor capable of executing functions described herein. The above examples are exemplary only, and are thus not necessarily intended to limit the definition and/or meaning of the term system controller. In the exemplary embodiment, the system controller **102** executes a set of instructions that are stored in one or more storage elements, memories, or modules in order to at least one of obtain and analyze detection data. Storage elements may be in the form of information sources or physical memory elements within the assay system **100**.

The set of instructions may include various commands that instruct the assay system **100** to perform specific operations such as the methods and processes of the various embodiments described herein. The set of instructions may be in the form of a software program. As used herein, the terms “software” and “firmware” are interchangeable, and include any computer program stored in memory for execution by a computer, including RAM memory, ROM memory, EPROM memory, EEPROM memory, and non-volatile RAM (NVRAM) memory. The above memory types are exemplary only, and are thus not limiting as to the types of memory usable for storage of a computer program.

The software may be in various forms such as system software or application software. Further, the software may be in the form of a collection of separate programs, or a program module within a larger program or a portion of a program module. The software also may include modular programming in the form of object-oriented programming. After obtaining the detection data, the detection data may be automatically processed by the assay system **100**, processed in response to user inputs, or processed in response to a request made by another processing machine (e.g., a remote request through a communication link).

The system controller **102** may be connected to the other components or sub-systems of the assay system **100** via communication links (indicated by dashed lines). The system controller **102** may also be communicatively connected to off-site systems or servers. The communication links may be hardwired or wireless. The system controller **102** may receive user inputs or commands, from the user interface **140**. The user input device **144** may include a keyboard, mouse, a touch-screen panel, and/or a voice recognition system, and the like. Alternatively or in addition, the user input device **144** may also be the display **142**.

FIG. 1 also illustrates a block diagram of the system controller **102**. In one embodiment, the system controller **102** includes one or more processors or modules that can communicate with one another. The system controller **102** is illustrated conceptually as a collection of modules, but may



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be implemented utilizing any combination of dedicated hardware boards, DSPs, processors, etc. Alternatively, the system controller **102** may be implemented utilizing an off-the-shelf PC with a single processor or multiple processors, with the functional operations distributed between the processors. As a further option, the modules described below may be implemented utilizing a hybrid configuration in which certain modular functions are performed utilizing dedicated hardware, while the remaining modular functions are performed utilizing an off-the-shelf PC and the like. The modules also may be implemented as software modules within a processing unit.

The system controller **102** may include a plurality of modules **151-158** that communicate with a system control module **150**. The system control module **150** may communicate with the user interface **140**. Although the modules **151-158** are shown as communicating directly with the system control module **150**, the modules **151-158** may also communicate directly with each other, the user interface **140**, or the other systems. Also, the modules **151-158** may communicate with the system control module **150** through the other modules.

The plurality of modules **151-158** include system modules **151-153** that communicate with the sub-systems. The fluidic control module **151** may communicate with the fluidic control system **134** to control the valves and flow sensors of the fluidic network **135** for controlling the flow of one or more fluids through the fluidic network **135**. The fluid storage module **152** may notify the user when fluids are low or when the waste reservoir must be replaced. The fluid storage module **152** may also communicate with the temperature control module **153** so that the fluids may be stored at a desired temperature.

The plurality of modules **151-158** may also include an image analysis module **158** that receives and analyzes the detection data (e.g., image data) from the detector assembly **108**. The processed detection data may be stored for subsequent analysis or may be transmitted to the user interface **140** to display desired information to the user. Protocol modules **155-157** communicate with the system control module **150** to control the operation of the sub-systems when conducting predetermined assay protocols. The protocol modules **155-157** may include sets of instructions for instructing the assay system **100** to perform specific operations pursuant to predetermined protocols.

The protocol module **155** may be configured to issue commands for generating a sample within the fluidic device **112**. For example, the protocol module **155** may direct the fluid storage system **136** and the temperature control system **138** to generate the sample in a sample area. In one particular embodiment, the protocol module **155** may issue commands to perform bridge PCR where clusters of clonal amplicons are formed on localized areas within a channel (or lane) of a flow cell.

The protocol module **156** may be a sequencing-by-synthesis (SBS) module configured to issue various commands for performing sequencing-by-synthesis processes. In some embodiments, the SBS module **156** may also process detection data. After generating the amplicons through bridge PCR, the SBS module **156** may provide instructions to linearize or denature the amplicons to make sstDNA and to add a sequencing primer such that the sequencing primer may be hybridized to a universal sequence that flanks a region of interest. Each sequencing cycle extends the sstDNA by a single base and is accomplished by modified DNA polymerase and a mixture of four types of nucleotides delivery of which can be instructed by the SBS module **156**.

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The different types of nucleotides have unique fluorescent labels, and each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in each cycle. After a single base is added to the sstDNA, the SBS module **156** may instruct a wash step to remove nonincorporated nucleotides by flowing a wash solution through the flow cell. The SBS module **156** may further instruct the excitation source assembly and detector assembly to perform an image session(s) to detect the fluorescence in each of the four channels (i.e., one for each fluorescent label). After imaging, the SBS module **156** may instruct delivery of a deblocking reagent to chemically cleave the fluorescent label and the terminator from the sstDNA. The SBS module **156** may instruct a wash step to remove the deblocking reagent and products of the deblocking reaction. Another similar sequencing cycle may follow. In such a sequencing protocol, the SBS module **156** may instruct the fluidic control system **134** to direct a flow of reagent and enzyme solutions through the fluidic device **112**.

In some embodiments, the SBS module **157** may be configured to issue various commands for performing the steps of a pyrosequencing protocol. Pyrosequencing detects the release of inorganic pyrophosphate (PPi) as particular nucleotides are incorporated into the nascent strand (Ronaghi, M. et al. (1996) "Real-time DNA sequencing using detection of pyrophosphate release." *Analytical Biochemistry* 242(1), 84-9; Ronaghi, M. (2001) "Pyrosequencing sheds light on DNA sequencing." *Genome Res.* 11(1), 3-11; Ronaghi, M. et al. (1998) "A sequencing method based on real-time pyrophosphate." *Science* 281(5375), 363; U.S. Pat. Nos. 6,210,891; 6,258,568 and 6,274,320, the disclosures of which are incorporated herein by reference in their entireties. In pyrosequencing, released PPi can be detected by being immediately converted to adenosine triphosphate (ATP) by ATP sulfurylase, and the level of ATP generated is detected via luciferase-produced photons. In this case, the fluidic device **112** may include millions of wells where each well has a single capture bead having clonally amplified sstDNA thereon. Each well may also include other smaller beads that, for example, may carry immobilized enzymes (e.g., ATP sulfurylase and luciferase) or facilitate holding the capture bead in the well. The SBS module **157** may be configured to issue commands to the fluidic control module **151** to run consecutive cycles of fluids that carry a single type of nucleotide (e.g., 1st cycle: A; 2nd cycle: G; 3rd cycle: C; 4th cycle: T; 5th cycle: A; 6th cycle: G; 7th cycle: C; 8th cycle: T; and on). When a nucleotide is incorporated into the DNA, pyrophosphate is released thereby instigating a chain reaction where a burst of light is generated. The burst of light may be detected by a sample detector of the detector assembly. Detection data may be communicated to the system control module **150**, the image analysis module **158**, and/or the SBS module **157** for processing. The detection data may be stored for later analysis or may be analyzed by the system controller **102** and an image may be sent to the user interface **140**.

In some embodiments, the user may provide user inputs through the user interface **140** to select an assay protocol to be run by the assay system **100**. In other embodiments, the assay system **100** may automatically detect the type of fluidic device **112** that has been inserted into the device holder **110** and confirm with the user the assay protocol to be run. Alternatively, the assay system **100** may offer a limited number of assay protocols that could be run with the determined type of fluidic device **112**. The user may select



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the desired assay protocol, and the assay system **100** may then perform the selected assay protocol based on preprogrammed instructions.

FIGS. **2** and **3** illustrate a workstation **160** formed in accordance with one embodiment that is configured for biological and chemical analysis of a sample. As shown, the workstation **160** is oriented with respect to mutually perpendicular X, Y, and Z-axes. In the illustrated embodiment, a gravitational force *g* extends parallel to the Z-axis. The workstation **160** may include a workstation casing **162** (or workstation housing) that is shown in phantom in FIGS. **2** and **3**. The casing **162** is configured to hold the various elements of the workstation **160**. For example, the workstation **160** may include similar elements as described above with respect to the assay system **100** (FIG. **1**). As shown, the workstation **160** has an optical deck **164** having a plurality of optical components mounted thereto. The optical components may be part of an optical assembly, such as the optical assembly **602** described with reference to FIG. **38** et al. The optical deck **164** may have a fixed position with respect to the casing **162**.

The workstation **160** may also include a sample deck **166** that is movably coupled to the optical deck **164**. The sample deck **166** may have a slidable platform **168** that supports a fluidic device thereon having a sample-of-interest. In the illustrated embodiment, the fluidic device is the fluidic device **300** that is described in greater detail below. The platform **168** is configured to slide with respect to the optical deck **166** and, more specifically, with respect to an imaging lens of the optical assembly **602**. To this end, the platform **168** may slide bi-directionally along the X-axis so that the fluidic device **300** may be positioned on the sample deck **166** and so that the imaging lens may slide over the fluidic device **300** to image the sample therein. In other embodiments, the platform **168** may be stationary and the sample deck **166** may slide bi-directionally along the X-axis to position the fluidic device **300** with respect to an imaging lens of the optical assembly **602**. Thus, the platform and sample deck can be moveable relative to each other due to movement of the sample deck, platform, or both.

Also shown, the workstation **160** may include a user interface **172**, a computing system **174** (FIG. **2**), and fluid storage units **176** and **178** (FIG. **4**). The user interface **172** may be a touchscreen that is configured to display information to a user and also receive user inputs. For example, the touchscreen may receive commands to perform predetermined assay protocols or receive inquiries from the user. The computing system **174** may include processors and modules, such as the system controller **102** and the modules **151-158** described with reference to FIG. **1**. The fluid storage units **176** and **178** may be part of a larger fluid storage system. The fluid storage unit **176** may be for collecting waste that results from performing the assays and the fluid storage unit **178** may include a buffer solution.

FIG. **4** is a diagram of a fluidic network **552** that may be used in the workstation **160** (FIG. **2**). As used herein, fluids may be liquids, gels, gases, or a mixture of thereof. Also, a fluid can be a mixture of two or more fluids. The fluidic network **552** may include a plurality of fluidic components (e.g., fluid lines, pumps, flow cells or other fluidic devices, manifolds, reservoirs) configured to have one or more fluids flowing therethrough. As shown, the fluidic network **552** includes a plurality of fluidic components **553-561** interconnected through fluid lines (indicated by the solid lines). In the illustrated embodiment, the fluidic network **552** includes a buffer solution container **553**, a reagent tray **554**, a multi-port valve **555**, a bypass valve **556**, a flow rate sensor

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**557**, a flow cell **558**, another flow rate sensor **559**, a pump **560**, and a waste reservoir **561**. Fluid flow directions are indicated by arrows along the fluid lines. In addition to the fluidic components **553-561**, the fluidic network may also include other fluidic components.

The reagent tray **554** may be similar to the reaction component tray (or reaction component storage unit) **1020** described in greater detail below. The tray **1020** may include various containers (e.g., vials or tubes) containing reaction components for performing assays with embodiments described herein. Operation of the multi-port valve **555** may be controlled by an assay system, such as the assay system **100**, to selectively flow different fluids, including mixtures thereof, to the flow cell **558**. The flow cell **558** may be the flow cell **200** or the fluidic device **300**, which are described in greater detail below, or other suitable fluidic devices.

FIGS. **5-60**, which are described in greater detail below, illustrate various elements (e.g., components, devices, assemblies, systems, and the like) and methods that may be used with the workstation **160**. These elements may cooperate with one another in imaging a sample, analyzing the detection data, and providing information to a user of the workstation **160**. However, the following elements and methods may also be used independently, in other apparatuses, or with other apparatuses. For example, the flow cell **200** and the fluidic device **300** may be used in other assay systems. The optical assembly **602** (and elements thereof) may be used for examining other items, such as microcircuits. Furthermore, the device holder **400** may be used to hold other fluidic devices, such as lab-on-chip devices. Assay systems with these devices may or may not include an optical assembly to detect the desired reactions.

FIGS. **5-7** illustrate a flow cell **200** formed in accordance with one embodiment. As shown in FIGS. **5-7**, the flow cell **200** is oriented relative to the X, Y, and Z-axes. The flow cell **200** is configured to hold a sample-of-interest **205** in a flow channel **206**. The sample **205** is illustrated as a plurality of DNA clusters that can be imaged during a SBS protocol, but other samples may be used in alternative embodiments. Although only the single U-shaped flow channel **206** is illustrated, alternative embodiments may include flow cells having multiple flow channels with differently shaped paths. The flow cell **200** may be in fluid communication with a fluidic system (not shown) that is configured to deliver reagents to the sample **205** in the flow channel **206**. In some embodiments, the sample **205** may provide detectable characteristics (e.g., through fluorescence or chemiluminescence) after desired reactions occur. For instance, the flow cell **200** may have one or more sample areas or regions (i.e., areas or regions where the sample **205** is located) from which optical signals are emitted. In some embodiments, the flow cell **200** may also be used to generate the sample **205** for performing a biological or chemical assay. For example, the flow cell **200** may be used to generate the clusters of DNA before the SBS protocol is performed.

As shown in FIGS. **5-7**, the flow cell **200** can include a first layer **202** and a second layer **204** that are secured together and define the flow channel **206** therebetween. The first layer **202** has a mounting surface **208** and an outer or exterior surface **210** (FIGS. **5** and **6**). The mounting and outer surfaces **208** and **210** face in opposite directions along the Z-axis and define a thickness  $T_1$  (FIGS. **5** and **6**) therebetween. The thickness  $T_1$  is substantially uniform along an XY-plane, but may vary in alternative embodiments. The second layer **204** has a channel surface **212** (FIG. **6**) and an outer or exterior surface **214**. The channel and



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outer surfaces **212** and **214** face in opposite directions along the Z-axis and define a thickness  $T_2$  (FIG. 6) therebetween.

Also shown in FIG. 5, the first layer **202** has a dimension or length  $L_1$  measured along the X-axis and another dimension or width  $W_1$  measured along the Y-axis. In some embodiments, the flow cell **200** may be characterized as a microdevice. Microdevices may be difficult to hold or move by an individual's hands. For example, the length  $L_1$  of the flow cell **200** may be about 100 mm, or about 50 mm, or less. In particular embodiments, the length  $L_1$  is about 30 mm or less. In some embodiments, the width  $W_1$  may be about 35 mm, or about 25 mm or less or, more particularly, the width  $W_1$  may be about 15 mm or less. Furthermore, a combined or total height  $H_T$  shown in FIG. 7 (e.g., a sum of thicknesses  $T_1$  and  $T_2$ ) may be about 10 mm, or about 5 mm or less. More specifically, the height  $H_T$  may be about 2 mm or about 1.5 mm or less.

The flow cell **200** includes edges **231-234** that are linear in the illustrated embodiment. Edges **231** and **233** are spaced apart by the width  $W_1$  and extend the length  $L_1$  of the flow cell **200**. Edges **232** and **234** are spaced apart by the length  $L_1$  and extend along the width  $W_1$ . Also shown, the second layer **204** may have a dimension or length  $L_2$  measured along the X-axis and another dimension or width  $W_2$  measured along the Y-axis. In the illustrated embodiment, the edges **231-234** define a perimeter of the flow cell **200** and extend along a common cell plane that extends parallel to the XY-plane. Also shown, the second layer **204** may have edges **241-244** that are similarly oriented as the edges **231-234** as shown in FIG. 5.

In the illustrated embodiment, the width  $W_1$  is substantially greater than the width  $W_2$ , and the second layer **204** is positioned on only a portion of the mounting surface **208**. As such, the mounting surface **208** includes exposed grip portions **208A** and **208B** on opposite sides of the second layer **204**. The width  $W_2$  extends between the grip portions **208A** and **208B**. The flow cell **200** may also have cell sides **256** and **258** that face in opposite directions along the Z-axis. In the illustrated embodiment, the cell side **256** includes the grip portions **208A** and **208B** and the exterior surface **214**, and the cell side **258** includes the exterior surface **210**. Also shown, the flow cell **200** may extend lengthwise between opposite first and second cell ends **246** and **248**. In the illustrated embodiment, the edges **232** and **242** are substantially flush with respect to each other at the first cell end **246**, and the edges **234** and **244** are substantially flush with respect to each other at the opposite second cell end **248**.

As shown in FIG. 6, the second layer **204** has at least one grooved portion **216** that extends along the channel surface **212**. In the illustrated embodiment, the channel surface **212** is etched to form the grooved portion **216**, but the grooved portion **216** may be formed by other processes, such as machining the channel surface **212**. To assemble the flow cell **200**, the channel surface **212** of the second layer **204** is mounted onto and secured to the mounting surface **208** of the first layer **202**. For example, the channel and mounting surfaces **212** and **208** may be bonded together using an adhesive (e.g., light-activated resin) that prevents leakage from the flow cell **200**. In other embodiments, the channel and mounting surfaces **212** and **208** may be secured together by other adhesives or mechanically interlocked and/or held together. Thus, the first layer **202** is configured to cover the grooved portion **216** of the second layer **204** to form the flow channel **206**. In the illustrated embodiment, the grooved portion **216** may be a single continuous groove that extends substantially the length  $L_2$  toward the first end, curves, and

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then extends substantially the length  $L_2$  toward the second end. Thus, the flow channel **206** may be substantially U-shaped.

In FIGS. 5-7 the sample **205** is shown as being located along only the mounting surface **208**. However, in other embodiments, the sample **205** may be located on any surface that defines the flow channel **206**. For instance, the sample **205** may also be located on the mating surface **212** of the grooved portion **216** that partially defines the flow channel **206**.

In the illustrated embodiment, the flow channel **206** may include a plurality of channel segments **250-252**. Different channel segments may have different dimensions with respect to the immediately upstream or downstream channel segment. In the illustrated embodiment, the flow channel **206** may include a channel segment **250**, which may also be referred to as the imaging segment **250**. The channel segment **250** may have a sample area that is configured to be imaged by an imaging system (not shown). The flow channel **206** may also have channel segments **251** and **252**, which may also be referred to as non-imaging segments **250** and **252**. As shown, the channel segments **250** and **252** extend parallel to each other through the flow cell **200**. The channel segments **251** and **252** of the flow channel **206** may be sized and shaped relative to the channel segment **250** to control the flow of fluid and gases that may flow therethrough.

For example, FIG. 7 also illustrates cross-sections  $C_1$ - $C_3$  of the channel segments **250-252**, respectively, that are taken perpendicular to a flow direction  $F_1$ . In some embodiments, the cross-sections  $C_1$ - $C_3$  may be differently sized (i.e., different cross-sectional areas) to control the flow of fluid through the flow channel **206**. For example, the cross-section  $C_1$  is greater in size than the cross-sections  $C_2$  and  $C_3$ . More specifically, the channel segments **250-252** of the flow channel **206** may have a substantially equal height  $H_1$  measured between the grooved portion **216** of the channel surface **212** (FIG. 6) and the mounting surface **208**. However, the channel segments **250-252** of the flow channel **206** may have different widths  $W_3$ - $W_5$ , respectively. The width  $W_3$  is greater than the widths  $W_4$  and  $W_5$ . The channel segment **251** may constitute a curved or elbow segment that fluidically joins the channel segments **250** and **252**. The cross-section  $C_3$  is smaller than the cross-sections  $C_1$  and  $C_2$ . For example, the width  $W_5$  is less than the widths  $W_3$  and  $W_4$ .

FIG. 8 is an enlarged view of the curved segment **251** and portions of the channel segments **250** and **252**. As described above, the channel segments **250** and **252** may extend parallel to each other. Within the flow channel **206**, it may be desirable to have a uniform flow across the sample area. For example, the fluid may include stream portions  $F_2$ - $F_4$ . Dimensions of the channel segments **250-252** may be configured so that the stream portions  $F_2$ - $F_4$  have substantially equal flow rates across the sample area. In such embodiments, different sections or portions of the sample **205** (FIG. 5) may have a substantially equal amount of time to react with reaction components within the fluid.

To this end, the curved segment **251** of the flow channel **206** may have a non-continuous contour that fluidically joins the channel segments **250** and **252**. For example, as shown in FIG. 8, the curved segment **251** may include a tapering portion **270**, an intermediate portion **276**, and a downstream portion **278**. As shown, the tapering portion **270** has a width  $W_{5,A}$  that gradually reduces in size. More specifically, the curved segment **251** may include sidewalls **272** and **274** that extend inward toward each other at a substantially equal angle. The intermediate portion **276** curves from the tapering



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portion 270 to the downstream portion 278. The intermediate portion 276 has a width  $W_{5B}$  that reduces in size and then begins to increase in size. The downstream portion 278 has a substantially uniform width  $W_{5C}$  throughout and extends along a substantially linear path from the intermediate portion 276 to the channel segment 252. In other words, the sidewalls 272 and 274 may extend parallel to each other throughout the downstream portion 278.

Returning to FIG. 7, the flow cell 200 includes inlet and outlet ports 222 and 224, respectively. The inlet and outlet ports 222 and 224 are formed only through the second layer 204. However, in alternative embodiments, the inlet and outlet ports 222 and 224 may be formed through only the first layer 202 or through both layers 202 and 204. The flow channel 206 is in fluid communication with and extends between the inlet and outlet ports 222 and 224. In particular embodiments, the inlet and outlet ports 222 and 224 are located proximate to each other at the cell end 248 of the flow cell 200 (or proximate to the edges 234 and 244). For example, a spacing 282 that separates the inlet and outlet ports 222 and 224 may be approximately equal to the width  $W_3$ . More specifically, the spacing 282 may be about 3 mm, about 2 mm, or less. Furthermore, the channel segments 250 and 252 may be separated by a spacing 280. The spacing 280 may be less than the width  $W_3$  of the channel segment 250 or, more particularly, less than the width  $W_4$  of the channel segment 252. Thus, a path of the flow channel 206 may be substantially U-shaped and, in the illustrated embodiment, have a non-continuous contour along the curved segment 251.

In alternative embodiments, the flow channel 206 may have various paths such that the inlet and outlet ports 222 and 224 have different locations in the flow cell 200. For example, the flow channel may form a single lane that extends from the inlet port at one end of the flow cell to the outlet port at the opposite end of the flow cell.

With respect to FIG. 6, in some embodiments, the thickness  $T_2$  (FIG. 6) of the second layer 204 is substantially uniform along the imaging portion 250. The uniform thickness  $T_2$  along the imaging portion 250 may be configured to transmit optical signals therethrough. Furthermore, the thickness  $T_1$  of the first layer 202 is substantially uniform along the imaging portion 250 and configured to permit uniform transfer of thermal energy therethrough into the flow channel 206.

FIGS. 9-11 illustrate a fluidic device 300 formed in accordance with one embodiment. For illustrative purposes, the fluidic device 300 is oriented with respect to the mutually perpendicular X, Y, and Z-axes shown in FIGS. 9 and 10. FIGS. 9 and 10 are perspective views of the fluidic device 300. As shown in FIGS. 9 and 10, the fluidic device 300 includes a cartridge (or flow cell carrier) 302 and the flow cell 200. The cartridge 302 is configured to hold the flow cell 200 and facilitate orienting the flow cell 200 for an imaging session.

In some embodiments, the fluidic device 300 and the cartridge 302 may be removable such that the cartridge 302 may be removed from an imaging system (not shown) by an individual or machine without damage to the fluidic device 300 or cartridge 302. For example, the cartridge 302 may be configured to be repeatedly inserted and removed into the imaging system without damaging the cartridge 302 or rendering the cartridge 302 unsuitable for its intended purpose. In some embodiments, the fluidic device 300 and the cartridge 302 may be sized and shaped to be handheld by an

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individual. Furthermore, the fluidic device 300 and the cartridge 302 may be sized and shaped to be carried by an automated system.

As shown in FIGS. 9 and 10, the cartridge 302 may include a housing or carrier frame 304 and a cover member 306 that is coupled to the housing 304. The housing 304 has housing or carrier sides 303 and 305 that face in opposite directions along the Z-axis and have a height H2 (shown in FIG. 11) extending therebetween. As shown in FIG. 9, the housing 304 includes a bridge member 324 at a loading end 316 of the fluidic device 300 and a base member 326 at an opposite receiving end 318 of the fluidic device 300. The housing 304 also includes a pair of spaced apart leg extensions 328 and 330 that extend between the bridge and base members 324 and 326. The bridge member 324 extends between and joins the leg extensions 328 and 330. The bridge member 324 may include a recess 321 (shown in FIG. 10) that opens to an exterior of the fluidic device 300. As shown in FIG. 9, the leg extensions 328 and 330 may have a plurality of grip members 371-374 that are configured to grip the cell side 256 of the flow cell 200.

Also shown in FIG. 9, the fluidic device 300 may have a device window 315 that passes entirely through the cartridge 302 along the Z-axis. In the illustrated embodiment, the device window 315 is substantially framed by the bridge member 324, the cover member 306, and the leg extensions 328 and 330. The device window 315 includes a reception space 308 and a plurality of recesses 320 and 322 that are immediately adjacent to the reception space 308. The reception space 308 is configured to receive the flow cell 200. When the flow cell 200 is positioned within the reception space 308, the flow cell 200 is exposed to an exterior of the fluidic device 300 such that the flow cell 200 may be viewed or directly engaged along the housing side 303 and also the housing side 305. For example, the cell side 258 (also shown in FIG. 11) that faces in an opposite direction along the Z-axis relative to the cell side 256. The cell side 256 may be viewed by the imaging system or directly engaged by another component along the housing side 303. Likewise, the cell side 258 may be viewed by the imaging system or directly engaged by another component along the housing side 305.

With respect to FIGS. 9 and 10, the cover member 306 may include a cover body 340 and a gasket 342 that are coupled to each other. The gasket 342 includes inlet and outlet passages 346 and 344 (shown in FIG. 9) that are located proximate to one another. In the illustrated embodiment, the cover body 340 and the gasket 342 are co-molded into a unitary structure. When formed, the cover body 340 and the gasket 342 may have different compressible properties. For example, in particular embodiments, the gasket 342 may comprise a material that is more compressible than material of the cover body 340. However, in alternative embodiments, the cover body 340 and the gasket 342 may be separate parts that are coupled together (e.g., mechanically or using an adhesive). In other embodiments, the cover body 340 and the gasket 342 may be different portions or regions of a single continuous structure.

The cover member 306 may be movably coupled to the housing 304. For example, the cover member 306 may be rotatably coupled to the base member 326 of the housing 304. In such embodiments, the gasket 342 is rotatable about an axis of rotation  $R_1$  between a mounted position (shown in FIG. 9) and a disengaged position (shown in FIG. 10). In other embodiments in which the cover member 306 is movably coupled to the housing 304, the cover member 306 may be detachable from the housing 304. For example,



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when attached to the housing 304, the detachable cover member may be in a mounted position that is similar to the mounted position as shown in FIG. 9. When unattached to the housing 304, the detachable cover member may be completely removed in a disengaged position.

Also shown in FIG. 10, the housing 304 may define a cartridge cavity 338 (FIG. 10) that is accessible when the cover member 306 is in the disengaged position. In some embodiments, an identification transmitter 336 may be positioned within the cartridge cavity 338. The identification transmitter 336 is configured to communicate information about the flow cell 200 to a reader. For example, the identification transmitter 336 may be an RFID tag. The information provided by the identification transmitter 336 may, for example, identify the sample in the flow cell 200, a lot number of the flow cell or sample, a date of manufacture, and/or the assay protocol to be performed when the flow cell 200 is inserted into the imaging system. The identification transmitter 336 may communicate other information as well.

FIG. 11 is a cross-section of the fluidic device 300 viewed along the Y-axis. In some embodiments, the reception space 308 is sized and shaped relative to the flow cell 200 so that the flow cell 200 is retained in the space, but in at least some configurations may float therein. As used herein, the term “float” and like terms includes the component being permitted to move a limited distance in at least one direction (e.g., along the X, Y, or Z-axes). For example, the flow cell 200 may be capable of shifting within the reception space 308 along the XY-plane. The flow cell 200 may also be capable of moving in a direction along the Z-axis within the reception space 308. Furthermore, the flow cell 200 can also be capable of slightly rotating within the reception space 308. In particular embodiments, the housing 304 permits the flow cell 200 to shift, move, and slightly rotate within the reception space 308 with respect to any of the X, Y, and Z-axes.

In some embodiments, the reception space 308 may also be characterized as the space that the fluidic device 300 allows the flow cell 200 to move freely within when the fluidic device 300 is holding the flow cell 200. Thus, dimensions of the reception space 308 may be based upon positions of reference surfaces of the fluidic device 300 that can directly engage the flow cell 200. The reference surfaces may be surfaces of the housing 304 or the cover member 306, including the gasket 342. For example, FIG. 11 illustrates a plurality of reference surfaces 381-387. The reference surfaces 381 and 382 of the grip members 371 and 372, respectively, and the reference surface 383 of the gasket 342 may limit movement of the flow cell 200 beyond a predetermined level when the flow cell 200 is held within the reception space 308. The reference surface 384 of the gasket 342 and the reference surface 385 of the bridge member 324 may limit movement of the flow cell 200 along the XY-plane. Furthermore, the reference surfaces 386 and 387 of the bridge member 324 and the cover member 306, respectively, may also limit movement of the flow cell 200 along the Z-axis. However, the reference surfaces 381-387 are exemplary only and the fluidic device 300 may have other reference surfaces that limit movement of the flow cell 200.

To assemble the fluidic device 300, the flow cell 200 may be loaded into the reception space 308. For example, the flow cell 200 may be advanced toward the device window 315 along the housing side 305. The edge 234 (FIG. 5) may be advanced between the grip members 372 and 373 and the gasket 342. The cell side 256 may then be rotated toward the grip members 371-374 so that the grip members 371-374

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interface the cell side 256. The edge 232 (FIG. 5) may then be moved toward the bridge member 324 and, more specifically, the reference surface 385 of the bridge member 324. In some embodiments, the bridge member 324 may be deflected or bent to provide more space for positioning the cell end 246 (FIG. 5) thereon. When the flow cell 200 is loaded into the cartridge 302, the housing 304 and the cover member 306 may effectively grip the perimeter of the flow cell 200 such that the flow cell 200 is confined to move only within the reception space 308.

In alternative embodiments, the cell end 246 may be first inserted positioned by the bridge member 324 and then the gasket 342. In other embodiments, the flow cell 200 may approach the housing side 303. The grip members 371-374 may have tapered or beveled surfaces that permit the flow cell 200 to be snapped into position within the reception space 308.

Before, after, or during the loading of the flow cell 200, the cover member 306 may be moved to the disengaged position so that the identification transmitter 336 (FIG. 10) may be positioned with the cartridge cavity 338 (FIG. 10). When the gasket 342 is in the mounted position, the inlet and outlet passages 346 and 344 may have a predetermined location and orientation with respect to the housing 304 and the reception space 308. The gasket 342 may be mounted over the flow cell 200 along an exposed portion of the flow cell 200 (i.e., the cell side 256). The inlet and outlet passages 346 and 344 may be generally aligned with the inlet and outlet ports 224 and 222 (FIG. 5).

However, it should be noted that the illustrated fluidic device 300 is only one particular embodiment, and the fluidic device 300 may have different configurations in alternative embodiments. For example, in alternative embodiments, the flow cell 200 may not be exposed to the exterior of the fluidic device 300 along each of the housing sides 303 and 305. Instead, the flow cell 200 may be exposed to the exterior along only one of the housing sides (e.g., the housing side 303). Furthermore, in alternative embodiments, the cover member 306 may not be rotatably coupled to the housing 304. For example, the cover member 306 may be entirely detachable.

FIGS. 12-15 illustrate fluidic devices 900 and 920 formed in accordance with alternative embodiments that may also be used in assay systems, such as the assay system 100 (FIG. 1) and the workstation 160 (FIG. 2). The fluidic devices 900 and 920 may include similar features as the fluidic device 300. For example, as shown, in FIGS. 12 and 13, the fluidic device 900 may include a cartridge (or flow cell carrier) 902 and the flow cell 200. The cartridge 902 is configured to hold the flow cell 200 and facilitate orienting the flow cell 200 for an imaging session. The cartridge 902 includes a housing 904 and a cover member 906 that is movably mounted to the housing 904. The cover member 906 is in the mounted position in FIG. 12 and the disengaged position in FIG. 13.

Also shown in FIGS. 12 and 13, the fluidic device 900 may include a sealing member 910 that covers the inlet and outlet ports 222 and 224 (FIG. 13) of the flow cell 200. In some embodiments, the sealing member 910 is configured to facilitate retaining fluid within the flow channel 206 so that the sample 205 (FIG. 5) within the flow channel 206 remains in a fluid environment. However, in some embodiments, the sealing member 910 may be configured to prevent unwanted materials from entering the flow channel 206. As shown in FIGS. 12 and 13, the sealing member 910 is a single piece of tape that extends between the cell ends 246 and 248 (FIG. 13). An overhang portion 912 may extend away from the cell end 246. In alternative embodiments, the sealing member



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910 may be more than one piece of tape (e.g., one piece of tape for each of the inlet and outlet ports 222 and 224) or the sealing member 910 may be other elements capable of covering the inlet and outlet ports 222 and 224. For example, the sealing member 910 could include plugs.

In some embodiments, the sealing member 910 covers the inlet and outlet ports 222 and 224 when the fluidic device 900 is not mounted to an assay system. For example, the sealing member 910 may be used when the fluidic device 900 is being stored or transported, or when a sample is being grown or generated within the flow cell 200. In such instances, the sealing member 910 may be secured to the flow cell 200 and the housing 904 as shown in FIG. 13. More specifically, the sealing member 910 may couple to and extend along the cell side 256 and cover the inlet and outlet ports 222 and 224. The sealing member 910 may also couple to a base member 914 of the housing 904. The cover member 906 may then be moved to the mounted position as shown in FIG. 12 such that the sealing member 910 is sandwiched between the inlet and outlet ports 222 and 224 and the cover member 906. The cover member 906 may facilitate preventing the sealing member 910 from being inadvertently removed. In alternative embodiments, the sealing member 910 may cover inlet and outlet passages 916 and 918 of the cover member 906.

FIGS. 14 and 15 illustrate the fluidic device 920, which may also have similar features as the fluidic devices 300 and 900. As shown, the fluidic device 920 includes a cartridge (or flow cell carrier) 922 and the flow cell 200. The cartridge 922 includes a housing 924 and a cover member 925 that is movably mounted to the housing 924. The cover member 925 is only shown in the mounted position in FIGS. 14 and 15. The housing 924 and the cover member 925 may be similar to the housings 204 and 904 and the cover member 306 and 906 described above.

However, the housing 924 may also include fin projections 926 and 928. The fin projections 926 and 928 are sized and shaped to be gripped by an individual or robotic device, such as when the fluidic device 920 is being inserted in or removed from a device holder (not shown). In some embodiments, the fin projections 926 and 928 may prevent the cover assembly (not shown) from moving to the closed position if the fluidic device 920 is not properly positioned. The fin projections 926 and 928 may include tactile features 927 and 929 that are configured to be gripped by the individual. In the illustrated embodiment, the fin projections 926 and 928 are located at a receiving end 930 of the fluidic device 920. The cover member 925 may extend between the fin projections 926 and 928. However, the fin projections 926 and 928 may have other locations along the cartridge 902.

FIGS. 16-24 show various features of a fluidic device holder 400 formed in accordance with one embodiment. FIG. 16 is a partially exploded view of the holder 400. When assembled, the holder 400 may be used to hold the fluidic device 300 (FIG. 9) and the flow cell 200 (FIG. 5) in a desired orientation during an imaging session. Furthermore, the holder 400 may provide an interface between the fluidic device 300 and the imaging system (not shown) in which the holder 400 may be configured to direct fluids through the flow cell 200 and provide or remove thermal energy from the flow cell 200. Although the holder 400 is shown as holding the fluidic device 300, the holder 400 may be configured to hold other fluidic devices, such as lab-on-chip devices or flow cells without cartridges.

As shown in FIG. 16, the holder 400 may include a removable cover assembly 404 and a support structure 402. In some embodiments, the holder 400 may also include a

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plate structure 406 and a movable platform 408. The plate structure 406 is operatively coupled to the cover assembly 404 and includes an opening 410 therethrough. Likewise, the platform 408 includes an opening 412 therethrough. The support structure 402 may include a heat sink 414 and a thermal module (or thermocycler) 416 that is mounted onto the heat sink 414. The thermal module 416 includes a base portion 418 and a pedestal 420. When the holder 400 is assembled, the support structure 402, the platform 408, and the plate structure 406 are stacked with respect to each other. As such, the opening 412 is sized and shaped to receive the base portion 418, and the opening 410 is sized and shaped to receive the pedestal 420. When assembled, the cover assembly 404 may be operatively coupled to the plate structure 406 and the support structure 402.

FIG. 17 shows the assembled holder 400. In the illustrated embodiment, a panel 424 is positioned over the plate structure 406 (FIG. 16). As shown in FIGS. 16 and 17, the cover assembly 404 includes a cover housing 435 that is coupled to the plate structure 406. The cover housing 435 may be substantially U-shaped having a pair of spaced apart housing legs 436 and 438 that extend in a common direction. The housing legs 436 and 438 may be rotatably coupled to the plate structure 406 at joints 437 and 439. The cover housing 435 may also include a bridge portion 440 that extends between and joins the housing legs 436 and 438. In this manner, the cover assembly 404 may be configured to provide a viewing space 442 (FIG. 17). The viewing space 442 may be sized and shaped to permit an imaging lens (not shown) to move in a direction Dx (FIG. 17) along and over the flow cell 200.

In the illustrated embodiment, the cover assembly 404 is movable relative to the plate structure 406 or support structure 402 between an open position (shown in FIG. 16) and a closed position (shown in FIG. 17). In the open position, the cover assembly 404 is withdrawn or retracted to permit access to a loading region 422 (shown in FIG. 18) of the holder 400 so that the fluidic device 300 may be removed from or inserted into the loading region 422. In the closed position, the cover assembly 404 is mounted over the fluidic device 300. In particular embodiments, the cover assembly 404 establishes a fluid connection with the fluidic device 300 in the closed position and presses the flow cell 200 against the support structure 402.

As shown in FIG. 16, in some embodiments, the holder 400 includes a coupling mechanism 450 to facilitate holding the cover assembly 404 in the closed position. For example, the coupling mechanism 450 may include an operator-controlled element 452 that includes a button 453 that is coupled to a pair of latch openings 456 and 458. The coupling mechanism 450 also includes a pair of latch ends 454 and 455 that project away from a mating face 460 of the cover housing 435. The cover housing 435 may be biased into the open position by spring elements 464 and 466. When the cover assembly 404 is moved into the closed position by an individual or machine, the latch ends 454 and 455 are inserted into the latch openings 456 and 458, respectively, and grip the operator-controlled element 452. To move the cover assembly 404 into the open position, the individual or machine may actuate the button 453 by, for example, pushing the button 453 inward. Since the cover housing 435 is biased by the spring elements 464 and 466, the cover housing 435 is rotated away from the panel 424 (FIG. 17) about the joints 437 and 439.

In alternative embodiments, the coupling mechanism 450 may include other elements to facilitate holding the cover assembly 404 in the closed position. For example, the latch



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ends **454** and **455** may be replaced by magnetic elements or elements that form an interference fit with openings.

FIG. **18** is an isolated perspective view of thermal module **416** and the heat sink **414** of the support structure **402**. The thermal module **416** may be configured to control a temperature of the flow cell **200** for predetermined periods of time. For example, the thermal module **416** may be configured to raise the temperature of the flow cell **200** so that DNA in the sample may denature. Furthermore, the thermal module **416** may be configured to remove thermal energy thereby lowering the temperature of the flow cell **200**. As shown, the pedestal **420** includes a base surface **430** that is sized and shaped to interface with the flow cell **200** (FIG. **5**). The base surface **430** faces in a direction along the Z-axis. The pedestal **420** may also include a plurality of alignment members **431-433** that are positioned around the base surface **430**. In the illustrated embodiment, the alignment members **431-433** have fixed positions with respect to the base surface **430**. The alignment members **431-433** have corresponding reference surfaces that are configured to engage the flow cell **200** and facilitate positioning the flow cell **200** for imaging. For example, the reference surfaces of the alignment members **431-433** may face in respective directions along the XY-plane and, as such, may be configured to limit movement of the flow cell **200** along the XY-plane. The support structure **402** may include at least a portion of the loading region **422**. The loading region **422** may be partially defined by the base surface **430** and the reference surfaces of the alignment members **431-433**.

FIGS. **19** and **20** illustrate an alignment assembly **470** that may be used with the holder **400** in accordance with one embodiment. FIG. **19** is a plan view of the holder **400** in which the cover housing **435** is shown in phantom to illustrate the alignment assembly **470**. FIG. **20** is a perspective view of the holder **400** in which the cover assembly **404** is in the open position. (In both FIGS. **19** and **20**, the panel **424** (FIG. **17**) has been removed for illustrative purposes.)

The fluidic device **300** is loaded into the loading region **422** in FIGS. **19** and **20**. When the fluidic device **300** is loaded, the flow cell **200** is placed onto the base surface **430** (FIG. **18**) and the alignment members **432**, **433**, and **431** are advanced through the recesses **320**, **322**, and **321** (FIGS. **9** and **10**) of the cartridge **302**. More specifically, the device window **315** (FIG. **9**) along the housing side **305** may be sized and shaped to be greater than a perimeter of the base surface **430**. As such, the cartridge **302** or housing **304** may be allowed to fall around the base surface **430**, but the flow cell **200** is prevented from falling by the base surface **430**. In this manner, the cell side **258** of the flow cell **200** may be pressed against the base surface **430** so that the thermal module **416** may control a temperature of the flow cell **200**. When the flow cell **200** is mounted on the base surface **430**, the reference surfaces **381-383** (FIG. **11**) of the cartridge **302** are pressed against the cell side **256** (FIG. **11**). At this time, a cell plane of the flow cell **200** that extends along the sample **205** may be substantially aligned with an object plane of the imaging system.

In the illustrated embodiment, when the fluidic device **300** is loaded into the loading region **422**, an identification reader of the assay system may detect information from the identification transmitter **336** (FIG. **10**). For example, the holder **400** may include an identification reader (not shown) in the plate structure **406** proximate to the identification transmitter **336**. The identification reading may occur before the cover assembly **404** is mounted onto the fluidic device **300**.

With reference to FIGS. **19** and **20**, the alignment assembly **470** includes various elements that cooperate together in

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orienting and positioning the flow cell **200** for imaging. For example, the alignment assembly **470** includes a movable locator arm **472** and an actuator **474** that is operatively coupled to the locator arm **472**. As shown, the actuator **474** includes a lever **476** and a pin element **478** that is coupled to the cover housing **435**. In the illustrated embodiment, the lever **476** is rotatable about an axis of rotation  $R_2$  (FIG. **19**). The lever **476** may be L-shaped having a first extension **480** configured to engage the pin element **478** and a second extension **482** configured to engage the locator arm **472**. The locator arm **472** is also rotatable about an axis of rotation  $R_3$  (FIG. **19**) and includes a finger **484** having an engagement end **486**. The alignment assembly **470** also includes a biasing element **490** (e.g., a coil spring) that engages the finger **484**. The engagement end **486** is configured to engage the cartridge **302** of the fluidic device **300**. In alternative embodiments, the engagement end **486** may be configured to directly engage the flow cell **200**.

The alignment assembly **470** is in an engaged arrangement in FIG. **19** and in a withdrawn arrangement in FIG. **20**. The locator arm **472** is in a retracted position when the alignment assembly **470** is in the withdrawn arrangement and in a biased position when the alignment assembly **470** is in the engaged arrangement. To align the flow cell **200** in the loading region **422**, the alignment assembly **470** is changed from the withdrawn arrangement to the engaged arrangement. For example, when the cover housing **435** is moved to the open position shown in FIG. **20**, the pin element **478** engages the first extension **480** of the lever **476** causing the lever **476** to rotate about the axis  $R_2$  in a counter-clockwise direction (as shown in FIG. **19**). The cover housing **435** may be maintained in the open position by the spring elements **464** and **466** (FIG. **16**). When the lever **476** is rotated, the second extension **482** rotates about the axis  $R_2$  and engages the locator arm **472**. The locator arm **472** is rotated about the axis  $R_3$  in a clockwise direction (as shown in FIG. **19**). When the locator arm **472** is rotated, the locator arm **472** is moved to the retracted position. When moved to the retracted position, the engagement end **486** is moved away from the reference surfaces of the alignment members **431-433**.

To change the alignment assembly **470** from the withdrawn arrangement to the engaged arrangement, the cover housing **435** may be rotated toward the fluidic device **300** and mounted over the flow cell **200**. When the cover housing **435** is moved toward the fluidic device **300**, the pin element **478** is rotated away from the first extension **480** of the lever **476**. When the second extension **482** moves away from the locator arm **472**, potential energy stored in the biasing element **490** may cause the locator arm **472** to rotate in a counter-clockwise direction such that the engagement end **486** presses against the cartridge **302**. As such, the locator arm **472** is moved to the biased position. When moved to the biased position, the engagement end **486** is moved toward the reference surfaces of the alignment members **431-433**.

FIG. **21** is an enlarged plan view of the fluidic device **300** in the loading region **422** when the engagement end **486** of the locator arm **472** is pressed against the cartridge **302**. The engagement end **486** may be configured to move within the XY-plane between the retracted and biased positions. When the engagement end **486** is moved toward the biased position and presses against the cartridge **302**, the engagement end **486** provides a force  $F_{XY}$  against the cartridge **302**. The cartridge **302** may shift along the XY-plane and/or press the flow cell **200** against the reference surfaces of the alignment members **431-433**. The force  $F_{XY}$  has an X-component and a Y-component. The X-component may press the flow cell



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200 against the alignment member 431, and the Y-component may press the flow cell 200 against the alignment members 432 and 433. As such, the alignment member 431 may stop movement of the flow cell 200 in a direction along the X-axis, and the alignment members 432 and 433 may stop movement of the flow cell 200 in a direction along the Y-axis.

Before the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 of the cover member 306 may be approximately aligned with the inlet and outlet ports 224 and 222 (FIG. 7), respectively, of the flow cell 200. After the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 are effectively (or operatively) aligned with the inlet and outlet ports 224 and 222 so that fluid may effectively flow therethrough.

Accordingly, the cover assembly 404 may be operatively coupled to the alignment assembly 470 such that one step or action causes the alignment assembly 470 to engage the fluidic device 300. More specifically, as the cover assembly 404 is mounted over the device in the closed position, the actuator 474 moves the locator arm 472 to the biased position. In the biased position, the locator arm 472 holds the flow cell 200 against the reference surfaces of the alignment members 431-433 in a fixed position along the XY-plane. When the cover assembly 404 is in the closed position, the viewing space 442 (FIG. 17) may be located over the flow cell 200 so that an imaging lens may move along the flow cell 200 to image the flow channel 206. As the cover assembly 404 is moved to the open position, the actuator 474 moves the locator arm 472 to the retracted position. However, in the illustrated embodiment, the flow cell 200 remains in position when the locator arm 472 is retracted. Accordingly, the flow cell 200 may be floatable relative to various elements. For example, the flow cell 200 may be floatable with respect to the cover member 306 and the gasket 342 when the cover member 306 is in the mounted position. The flow cell 200 may also be floatable relative to the cover assembly 404 and the base surface 430.

In some embodiments, the alignment assembly 470 and the cover assembly 404 may operate at a predetermined sequence. For example, in particular embodiments, the locator arm 472 is configured to hold the flow cell 200 against the alignment members 431-433 in the fixed position before the cover assembly 404 reaches the closed position. When the cover assembly 404 reaches the closed position, the cover assembly 404 may facilitate pressing the flow cell 200 against the base surface 430 and also pressing the inlet and outlet passages 346 and 344 against the inlet and outlet ports 224 and 222. Generally, the alignment assembly 470 can be configured to position the flow cell 200 in the x and y dimensions after the base surface 430 positions the flow cell 200 in the z dimension. Alternatively, an alignment assembly can be configured to position the flow cell 200 first in the x and y dimensions and then in the z dimension. Thus, alignment in the x, y and z dimensions can occur sequentially and in various orders in response to a single step or motion carried out by a user.

In alternative embodiments, the alignment assembly 470 may not be operatively coupled to the cover assembly 404 as described above. Instead, the alignment assembly 470 and the cover assembly 404 may operate independently from each other. As such, an individual may be required to perform a plurality of steps to align the flow cell 200 and fluidically couple the flow cell 200. For example, the alignment assembly 470 can be separately actuated by an individual thereby moving the locator arm 472 to align the flow

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cell 200. After the flow cell 200 is aligned, the individual may then lower the cover assembly 404 onto the flow cell 200. Furthermore, the alignment assembly 470 may comprise additional and/or other components than those described above.

FIG. 22 is an isolated perspective view of the cover assembly 404 in the closed position. FIG. 22 illustrates dimensions of the viewing space 442. As shown, the cover housing 435 may have a top surface 492. The viewing space 442 may have a depth  $D_p$  that is measured from the top surface 492 to the fluidic device 300 or the flow cell 200. The viewing space 442 may also have a width  $W_g$  measured along the Y-axis and a length  $L_g$  measured along the X-axis. The dimensions of the viewing space 442 may be sized so that an imaging lens (not shown) may move therethrough over the flow cell 200. More specifically, an imaging lens may enter the viewing space 442 through an access opening 443 and move in a direction along the X-axis over the flow cell 200.

FIG. 23 is a cross-section of the cover assembly 404 taken along the line 23-23 in FIG. 22. In the illustrated embodiment, the cover assembly 404 may include a plurality of compression arms 494 and 496. The compression arms 494 and 496 are configured to provide respective compressive forces  $F_{C1}$  and  $F_{C2}$  against the housing side 303 of the fluidic device 300. In the illustrated embodiment, the compression arms 494 and 496 press against the cartridge 302. However, in alternative embodiments, the compression arms 494 and 496 may press against the flow cell 200.

The compressive forces  $F_{C1}$  and  $F_{C2}$  press the housing 304 of the fluidic device 300 thereby pressing the cell side 256 (FIG. 9) of the flow cell 200 against the thermal module 416. As such, the flow cell 200 may maintain intimate contact with the base surface 430 for transferring thermal energy therebetween. In the illustrated embodiment, the compression arms 494 and 496 operate independently of each other. For example, each of the compression arms 494 and 496 is operatively coupled to respective compression springs 495 and 497.

As shown in FIG. 23, the compression arms 494 and 496 extend toward the viewing space 442 and the loading region 422. The compression arms 494 and 496 may engage the housing side 303 when the cover assembly 404 is moved to the closed position. As the compression arms 494 and 496 press against the housing side 303, resistance from the housing side 303 may cause the compression arms 494 and 496 to rotate about axes  $R_4$  and  $R_5$ . Each of the compression springs 495 and 497 may resist the rotation of the respective compression arm thereby providing the corresponding compressive force  $F_C$  against the housing side 303. Accordingly, the compression arms 494 and 496 are independently biased relative to each other.

FIG. 24 is an isolated perspective view of a flow assembly 500 of the cover assembly 404 (FIG. 16). The flow assembly 500 includes a manifold body 502 and upstream and downstream flow lines 504 and 506. As shown in FIG. 16, the manifold body 502 may extend between the housing legs 436 and 438. Returning to FIG. 24, the flow lines 504 and 506 are mechanically and fluidically coupled to the manifold body 502 at body ports 508 and 510, respectively. The flow lines 504 and 506 also include line ends 514 and 516 that are configured to be inserted into the inlet and outlet passages 346 and 344 of the gasket 342.

As shown in FIG. 24, the flow assembly 500 is in a mounted position with respect to the gasket 342. In the mounted position, the line ends 514 and 516 are inserted into the inlet and outlet passages 346 and 344, respectively, so



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that fluid may flow through the flow cell **200**. Furthermore, in the mounted position, the flow assembly **500** may press the gasket **342** (FIG. 9) against the flow cell **200** so that the fluid connection is effectively sealed. To this end, the flow assembly **500** may include biasing springs **520** and **522**. The biasing springs **520** and **522** are configured to press against an interior of the cover housing **435** (FIG. 16) and provide a force  $F_{C3}$  against the gasket **342**. The coupling mechanism **450** (FIG. 16) may facilitate maintaining the seal against the gasket **342**.

Accordingly, the cover assembly **404** may press against the housing **304** of the fluidic device **300** at three separate compression points. More specifically, the gasket **342** may constitute a first compression point  $P_1$  (shown in FIG. 24) when engaged by the line ends **514** and **516**, and the compression arms **494** and **496** may contact the fluidic device **300** at second and third compression points  $P_2$  and  $P_3$  (shown in FIG. 23). As shown in FIGS. 22-24, the three compression points  $P_1$ - $P_3$  are distributed about the flow cell **200**. Moreover, the cover assembly **404** independently provides the compressive forces  $F_{C1}$ - $F_{C3}$  at the compression points  $P_1$ - $P_3$ . As such, the cover assembly **404** may be configured to provide a substantially uniform compressive force against the fluidic device **300** so that the flow cell **200** is uniformly pressed against the base surface **430** and the fluidic connection is sealed from leakage.

FIG. 25 is a block diagram of a method **530** of positioning a fluidic device for sample analysis. The method **530** includes positioning at **532** a removable fluidic device on a base surface. The fluidic device may be similar to the fluidic device **300** described above. For example, the fluidic device may include a reception space, a flow cell located within the reception space, and a gasket. The flow cell may extend along an object plane in the reception space and be floatable relative to the gasket within the object plane. The method **530** also includes moving the flow cell at **534** within the reception space while on the base surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket. The moving operation **534** may include actuating a locator arm to press the flow cell against alignment members.

FIG. 26 is a block diagram illustrating a method **540** of positioning a fluidic device for sample analysis. The fluidic device **300** may be similar to the fluidic device **300** described above. The method **540** includes providing a fluidic device at **542** having a device housing that includes a reception space and a floatable flow cell located within the reception space. The device housing may include recesses that are located immediately adjacent to the reception space. The method also includes positioning at **544** the fluidic device on a support structure having alignment members. The alignment members may be inserted through corresponding recesses. Furthermore, the method **540** may include moving the flow cell at **546** within the reception space. When the flow cell is moved within the reception space, the alignment members may engage edges of the flow cell. The moving operation **546** may include actuating a locator arm to press the flow cell against the alignment members.

FIG. 27 is a block diagram illustrating a method **550** for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes. The method **550** includes providing an alignment assembly at **552**. The alignment assembly may be similar to the alignment assembly **470** described above. More specifically, the alignment assembly may include a movable locator arm that has an engagement end. The locator arm may be movable between retracted and

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biased positions. The method **550** also includes positioning a fluidic device at **554** on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. Furthermore, the method **550** may include moving at **556** the locator arm to the biased position. The locator arm can press the device against the reference surfaces such that the device is held in a fixed position.

FIGS. 28-37 illustrate various features of a fluid storage system **1000** (FIG. 28). The storage system **1000** is configured to store and regulate a temperature of various fluids that may be used during predetermined assays. The storage system **1000** may be used by the workstation **160** (FIG. 2) and enclosed by the casing **162** (FIG. 3). As shown in FIG. 28, the storage system **1000** includes an enclosure **1002** having a base shell (or first shell) **1004** and a top shell (or second shell) **1006** that are coupled together and define a system cavity **1008** therebetween. The enclosure **1002** may also include a system door **1010** that is configured to open and provide access to the system cavity **1008**. Also shown, the storage system **1000** may include a temperature-control assembly **1012** that is coupled to a rear of the enclosure **1002** and an elevator drive motor **1014** that is located on the top shell **1006**.

FIG. 29 is a side cross-section of the storage system **1000** and illustrates the system cavity **1008** in greater detail. The storage system **1000** may also include a reaction component tray (or reaction component storage unit) **1020** and a fluid removal assembly **1022** that includes an elevator mechanism **1024**. The tray **1020** is configured to hold a plurality of tubes or containers for storing fluids. The elevator mechanism **1024** includes the drive motor **1014** and is configured to move components of the removal assembly **1022** bi-directionally along the Z-axis. In FIG. 29, the tray **1020** is located in a fluid-removal position such that fluid held by the tray **1020** may be removed and delivered to, for example, a fluidic device for performing a desired reaction or for flushing the flow channels of the fluidic device.

Also shown, the temperature-control assembly **1012** may project into the system cavity **1008**. The temperature-control assembly **1012** is configured to control or regulate a temperature within the system cavity **1008**. In the illustrated embodiment, the temperature-control assembly **1012** includes a thermo-electric cooling (TEC) assembly.

FIG. 30 is a perspective view of the removal assembly **1022**. As shown, the removal assembly **1022** may include a pair of opposing guide rails **1032** and **1034**. The opposing guide rails **1032** and **1034** are configured to receive and direct the tray **1020** to the fluid-removal position shown in FIG. 29. The guide rails **1032** and **1034** may include projected features or ridges **1035** that extend longitudinally along the guide rails **1032** and **1034**. The guide rails **1032** and **1034** are configured to be secured to the base shell **1004** (FIG. 28). The removal assembly **1022** also includes support beams (or uprights) **1036** and **1038** that extend in a direction along the Z-axis. A guide plate **1040** of the removal assembly may be coupled to the support beams **1036** and **1038** at an elevated distance  $D_z$  and project therefrom along the XY-plane. In the illustrated embodiment, the guide plate **1040** is affixed to the support beams **1036** and **1038**.

The elevator mechanism **1024** includes structural supports **1041** and **1042**, a lead screw **1044** that extends between the structural supports **1041** and **1042**, and a stage assembly **1046** that includes a transport platform **1048**. The structural supports **1041** and **1042** are secured to opposite ends of the support beams **1036** and **1038** and are configured to support the elevator mechanism **1024** during operation.



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Threads of the lead screw **1044** are operatively coupled to the stage assembly **1046** such that when the lead screw **1044** is rotated, the stage assembly **1046** moves in a linear direction along the Z-axis (indicated by the double arrows).

The transport platform **1048** is configured to hold an array of sipper tubes **1050**. The sipper tubes **1050** may be in fluid communication with a system pump (not shown) that is configured to direct a flow of fluid through the sipper tubes **1050**. As shown, the sipper tubes **1050** include distal portions **1052** that are configured to be inserted into component wells **1060** (shown in FIG. 31) of the tray **1020**. The distal portions **1052** extend through corresponding openings **1053** of the guide plate **1040**.

The elevator mechanism **1024** is configured to move the sipper tubes **1050** between withdrawn and deposited levels. At the deposited level (shown in FIGS. 50 and 51), the distal portions **1052** of the sipper tubes **1050** are inserted into the component wells **1060** to remove fluid therefrom. At the withdrawn level, the distal portions **1052** are completely removed from the tray **1020** such that the tray **1020** may be removed from the system cavity **1008** (FIG. 28) without damage to the sipper tubes **1050** or the tray **1020**. More specifically, when the drive motor **1014** rotates the lead screw **1044**, the stage assembly **1046** moves along the Z-axis in a direction that is determined by a rotational direction of the lead screw **1044**. Consequently, the transport platform **1048** moves along the Z-axis while holding the sipper tubes **1050**. If the transport platform **1048** advances toward the guide plate **1040**, the distal portions **1052** slide through the corresponding openings **1053** of the guide plate **1040** toward the tray **1020**. The guide plate **1040** is configured to prevent distal portions **1052** from becoming misaligned with the component wells **1060** before the distal portions **1052** are inserted therein. When the elevator mechanism **1024** moves the stage assembly **1046** away from the guide plate **1040**, a distance ( $\Delta Z$ ) between the transport platform **1048** and the guide plate **1040** increases until the distal portions **1052** are withdrawn from the component wells **1060** of the tray **1020**.

FIG. 30 illustrates additional features for operating the elevator mechanism **1024**. For example, the stage assembly **1046** may also include a guide pin **1058** (also shown in FIG. 29) that is affixed to and extends from the transport platform **1048** in a direction that is parallel to the sipper tubes **1050**. The guide pin **1058** also extends through a corresponding opening **1053** of the guide plate **1040**. In the illustrated embodiment, the guide pin **1058** extends a greater distance than the sipper tubes **1050** so that the guide pin **1058** reaches the tray **1020** before the sipper tubes **1050** are inserted into the component wells **1060**. Thus, if the tray **1020** is misaligned with respect to the sipper tubes **1050**, the guide pin **1058** may engage the tray **1020** and adjust the position of the tray **1020** so that the component wells **1060** are properly aligned with the corresponding sipper tubes **1050** before the sipper tubes **1050** are inserted therein.

In addition to the above, the removal assembly **1022** may include a position sensor **1062** and a location sensor (not shown). The position sensor **1062** is configured to receive a flag **1063** (shown in FIG. 34) of the tray **1020** to determine that the tray **1020** is present in the system cavity **1008** (FIG. 28) and at least approximately aligned for receiving the sipper tubes **1050**. The location sensor may detect a flag **1064** of the stage assembly **1046** to determine a level of the stage assembly **1046**. If the flag **1064** has not reached a threshold level along the Z-axis, the location sensor may communicate with the workstation **160** (or other assay system) to notify the user that the tray **1020** is not ready for

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removal. The workstation **160** could also prevent the user from opening the system door **1010**.

Furthermore, when the distal portions **1052** of the sipper tubes **1050** are initially inserted into the component wells **1060**, the sipper tubes **1050** may pierce protective foils that cover the component wells **1060**. In some instances, the foils may grip the sipper tubes **1050**. When the sipper tubes **1050** are subsequently withdrawn from the corresponding component wells **1060**, the gripping of the protective foils may collectively lift the tray **1020**. However, in the illustrated embodiment, the ridges **1035** are configured to grip a tray base **1070** (FIG. 31) and prevent the tray base **1070** from being lifted in a direction along the Z-axis. For example, the ridges **1035** may grip a lip **1071** of the tray base **1070**.

FIGS. 31-34 illustrate different views of the tray **1020**. The tray **1020** is configured to hold a plurality of component wells **1060**. The component wells **1060** may include various reaction components, such as, but not limited to, one or more samples, polymerases, primers, denaturants, linearization mixes for linearizing DNA, enzymes suitable for a particular assay (e.g., cluster amplification or SBS), nucleotides, cleavage mixes, oxidizing protectants, and other reagents. In some embodiments, the tray **1020** may hold all fluids that are necessary to perform a predetermined assay. In particular embodiments, the tray **1020** may hold all reaction components necessary for generating a sample (e.g., DNA clusters) within a flow cell and performing sample analysis (e.g., SBS). The assay may be performed without removing or replacing any of the component wells **1060**.

The component wells **1060** include rectangular component wells **1060A** (shown in FIGS. 35-36) and tubular component wells **1060B** (shown in FIG. 37). The tray **1020** includes a tray base **1070** and a tray cover **1072** coupled to the tray base **1070**. As shown in FIGS. 31 and 32, the tray cover **1072** includes a handle **1074** that is sized and shaped to be gripped by a user of the tray **1020**. The tray cover **1072** may also include a grip recess **1076** that is sized and shaped to receive one or more fingers of the user.

As shown in FIGS. 31 and 32, the tray cover **1072** may include a plurality of tube openings **1080** that are aligned with corresponding component wells **1060**. The tube openings **1080** may be shaped to direct the sipper tubes **1050** (exemplary sipper tubes **1050** are shown in FIG. 31) into the corresponding component wells **1060**. As shown in FIG. 32, the tray cover **1072** also includes a pin opening **1082** that is sized and shaped to receive the guide pin **1058**. The guide pin **1058** is configured to provide minor adjustments to the position of the tray **1020** if the guide pin **1058** approaches and enters the pin opening **1082** in a misaligned manner. Also shown, the tray **1020** may include an identification tag **1084** along a surface of the tray cover **1072**. The identification tag **1084** is configured to be detected by a reader to provide the user with information regarding the fluids held by the component wells **1060**.

As shown in FIGS. 33 and 34, the tube openings **1080** are at least partially defined by rims **1086** that project from a surface **1073** of the tray cover **1072**. The rims **1086** project a small distance away from the surface **1073** to prevent inadvertent mixing of fluids that are accidentally deposited onto the tray cover **1072**. Likewise, the identification tag **1084** may be attached to a raised portion **1088** of the tray cover **1072**. The raised portion **1088** may also protect the identification tag **1084** from inadvertently contacting fluids.

FIG. 35 shows a side cross-sectional view of the component well **1060A**, and FIG. 36 shows a bottom perspective view of the component well **1060A**. As shown, the component well **1060A** includes opposite first and second ends



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**1091** and **1092** and a reservoir **1090** (FIG. **35**) extending therebetween. The reservoir **1090** has a depth  $D_R$  (FIG. **35**) that increases as the reservoir **1090** extends from the second end **1092** to the first end **1091**. The component well **1060A** is configured to receive the sipper tube **1050** in a deeper portion of the reservoir **1090**. As shown in FIG. **36**, the component well **1060A** includes a plurality of projections **1094** along an exterior surface that are configured to rest upon a surface of the tray base **1070**.

FIG. **37** is a perspective view of the component well **1060B**. As shown, the component well **1060B** may also include a plurality of projections **1096** around an exterior surface of the component well **1060B**. The component well **1060B** extends along a longitudinal axis **1097** and has a profile that tapers as the component well **1060B** extends longitudinally to a bottom **1098**. The bottom **1098** may have a substantially planar surface.

FIG. **61** illustrates a method **960** for performing an assay for biological or chemical analysis. In some embodiments, the assay may include a sample generation protocol and a sample analysis protocol. For example, the sample generation protocol may include generating clusters of DNA through bridge amplification and the sample analysis protocol may include sequencing-by-synthesis (SBS) analysis using the clusters of DNA. The sample generation and sample analysis operations may be conducted within a common assay system, such as the assay system **100** or the workstation **160**, and without user intervention between the operations. For instance, a user may be able to load a fluidic device into the assay system. The assay system may automatically generate a sample for analysis and carry out the steps for performing the analysis.

With respect to FIG. **61**, the method **960** includes establishing at **962** a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components. The reaction components may be configured for conducting one or more assays. The fluidic device may be, for example, the fluidic device **300** or the flow cell **200** described above. In some embodiments, the sample area includes a plurality of reaction components (e.g., primers) immobilized thereon. The storage unit may be, for example, the storage unit **1020** described above. The reaction components may include sample-generation components that are configured to be used to generate the sample, and sample-analysis components that are configured to be used to analyze the sample. In particular embodiments, the sample-generation components include reaction components for performing bridge amplification as described above. Furthermore, in particular embodiments, the sample-analysis components include reaction components for performing SBS analysis as described above.

The method **960** also includes generating at **964** a sample at the sample area of the fluidic device. The generating operation **964** may include flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. For example, a thermocycler may be used to facilitate hybridizing nucleic acids. However, isothermal methods can be used if desired. Furthermore, a flow rate of the fluids may be controlled to permit hybridization or other desired chemical reactions. In particular embodiments, the generating operation **964** includes conducting multiple bridge-amplification cycles to generate a cluster of DNA.

An exemplary protocol for bridge amplification can include the following steps. A flow cell is placed in fluid communication with a reaction component storage unit. The

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flow cell includes one or more surfaces to which are attached pairs of primers. A solution having a mixture of target nucleic acids of different sequences is contacted with a solid support. The target nucleic acids can have common priming sites that are complementary to the pairs of primers on the flow cell surface such that the target nucleic acids bind to a first primer of the pairs of primers on the flow cell surface. An extension solution containing polymerase and nucleotides can be introduced to the flow cell such that a first amplification product, which is complementary to the target nucleic acid, is formed by extension of the first primer. The extension solution can be removed and replaced with a denaturation solution. The denaturation solution can include chemical denaturants such as sodium hydroxide and/or formamide. The resulting denaturation conditions release the original strand of the target nucleic acid, which can then be removed from the flow cell by removing the denaturation solution and replacing it with the extension solution. In the presence of the extension solution the first amplification product, which is attached to the support, can then hybridize with a second primer of the primer pairs attached to the flow cell surface and a second amplification product comprising an attached nucleic acid sequence complementary to the first amplification product can be formed by extension of the second primer. Repeated delivery of the denaturation solution and extension solution can be used to form clusters of the target nucleic acid at discrete locations on the surface of the flow cell. Although the above protocol is exemplified using chemical denaturation, it will be understood that thermal denaturation can be carried out instead albeit with similar primers and target nucleic acids. Further description of amplification methods that can be used to produce clusters of immobilized nucleic acid molecules is provided, for example, in U.S. Pat. No. 7,115,400; U.S. Publication No. 2005/0100900; WO 00/18957; or WO 98/44151, each of which is incorporated by reference herein.

The method **960** also includes analyzing at **966** the sample at the sample area. Generally, the analyzing operation **966** may include detecting any detectable characteristic at the sample area. In particular embodiments, the analyzing operation **966** includes flowing at least one sample-analysis component to the sample area. The sample-analysis component may react with the sample to provide optically detectable signals that are indicative of an event-of-interest (or desired reaction). For example, the sample-analysis components may be fluorescently-labeled nucleotides used during SBS analysis. When excitation light is incident upon the sample having fluorescently-labeled nucleotides incorporated therein, the nucleotides may emit optical signals that are indicative of the type of nucleotide (A, G, C, or T), and the imaging system may detect the optical signals.

A particularly useful SBS protocol exploits modified nucleotides having removable 3' blocks, for example, as described in WO 04/018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. Repeated cycles of SBS reagents can be delivered to a flow cell having target nucleic acids attached thereto, for example, as a result of the bridge amplification protocol set forth above. The nucleic acid clusters can be converted to single stranded form using a linearization solution. The linearization solution can contain, for example, a restriction endonuclease capable of cleaving one strand of each cluster. Other methods of cleavage can be used as an alternative to restriction enzymes or nicking enzymes, including inter alia chemical cleavage (e.g., cleavage of a diol linkage with periodate), cleavage of abasic sites by cleavage with endonuclease (for example 'USER', as supplied by NEB, Ips-



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wich, Mass., USA, part number M5505S), by exposure to heat or alkali, cleavage of ribonucleotides incorporated into amplification products otherwise comprised of deoxyribonucleotides, photochemical cleavage or cleavage of a peptide linker. After the linearization step a sequencing primer can be delivered to the flow cell under conditions for hybridization of the sequencing primer to the target nucleic acids that are to be sequenced.

The flow cell can then be contacted with an SBS extension reagent having modified nucleotides with removable 3' blocks and fluorescent labels under conditions to extend a primer hybridized to each target nucleic acid by a single nucleotide addition. Only a single nucleotide is added to each primer because once the modified nucleotide has been incorporated into the growing polynucleotide chain complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase cannot add further nucleotides. The SBS extension reagent can be removed and replaced with scan reagent containing components that protect the sample under excitation with radiation. Exemplary components for scan reagent are described in US publication US 2008/0280773 A1 and U.S. Ser. No. 13/018,255, each of which is incorporated herein by reference. The extended nucleic acids can then be fluorescently detected in the presence of scan reagent. Once the fluorescence has been detected, the 3' block may be removed using a deblock reagent that is appropriate to the blocking group used. Exemplary deblock reagents that are useful for respective blocking groups are described in WO04018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. The deblock reagent can be washed away leaving target nucleic acids hybridized to extended primers having 3' OH groups that are now competent for addition of a further nucleotide. Accordingly the cycles of adding extension reagent, scan reagent, and deblock reagent, with optional washes between one or more of the steps, can be repeated until a desired sequence is obtained. The above cycles can be carried out using a single extension reagent delivery step per cycle when each of the modified nucleotides has a different label attached thereto, known to correspond to the particular base. The different labels facilitate discrimination between the bases added during each incorporation step. Alternatively, each cycle can include separate steps of extension reagent delivery followed by separate steps of scan reagent delivery and detection, in which case two or more of the nucleotides can have the same label and can be distinguished based on the known order of delivery.

Continuing with the example of nucleic acid clusters in a flow cell, the nucleic acids can be further treated to obtain a second read from the opposite end in a method known as paired end sequencing. Methodology for paired end sequencing are described in PCT publication WO07010252, PCT application Serial No. PCTGB2007/003798 and US patent application publication US 2009/0088327, each of which is incorporated by reference herein. In one example, a series of steps may be performed as follows; generate clusters as set forth above, linearize as set forth above, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above, "invert" the target nucleic acids on the flow cell surface by synthesizing a complementary copy, linearize the resynthesized strand, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above. The inversion step can

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be carried out by delivering reagents as set forth above for a single cycle of bridge amplification.

Although the analyzing operation has been exemplified above with respect to a particular SBS protocol, it will be understood that other protocols for sequencing any of a variety of other molecular analyses can be carried out as desired. Appropriate modification of the apparatus and methods to accommodate various analyses will be apparent in view of the teaching set forth herein and that which is known about the particular analysis method.

In some embodiments, the method 960 is configured to be conducted with minimal user intervention. The generating and analyzing operations 964 and 966 may be conducted in an automated manner by an assay system. For example, in some cases, a user may only load the fluidic device and the storage unit and activate the assay system to perform the method 960. In some embodiments, during the generating and analyzing operations 964 and 966, the storage unit and the fluidic device remain in fluid communication from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. In other words, the fluidic device and the storage unit may remain in fluid communication from before the sample is generated until after the sample is analyzed. In some embodiments, the fluidic device is continuously held by the device holder from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. During such time, the device holder and an imaging lens may be automatically moved with respect to each other. The storage unit and the fluidic device may remain in fluid communication when the fluidic device and the imaging lens are automatically moved with respect to each other. In some embodiments, the assay system is contained within a workstation housing and the generating and analyzing operations 964 and 966 are conducted exclusively within the workstation housing.

FIG. 38 is a schematic illustration of an optical imaging system 600 formed in accordance with one embodiment. The imaging system 600 includes an optical assembly 602, a light source (or excitation light) module or assembly 604, a flow cell 606 having a sample area 608, and imaging detectors 610 and 612. The light source module 604 includes first and second excitation light sources 614 and 616 that are configured to illuminate the sample area 608 with different excitation spectra. In particular embodiments, the first and second excitation light sources 614 and 616 comprise first and second semiconductor light sources (SLSs). SLSs may include light-emitting diodes (LEDs) or laser diodes. However, other light sources may be used in other embodiments, such as lasers or arc lamps. The first and second SLSs may have fixed positions with respect to the optical assembly 602.

As shown, the optical assembly 602 may include a plurality of optical components. For example, the optical assembly 602 may include lenses 621-627, emission filters 631-634, excitation filters 635 and 636, and mirrors 641-645. The plurality of optical components are arranged to at least one of (a) direct the excitation light toward the sample area 608 of the flow cell 606 or (b) collect emission light from the sample area 608. Also shown, the imaging system 600 may also include a flow system 652 that is in fluid communication with the flow cell 606 and a system controller 654 that is communicatively coupled to the first and second excitation light sources 614 and 616 and the flow system 652. The controller 654 is configured to activate the



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flow system **652** to flow reagents to the sample area **608** and activate the first and second SLSs after a predetermined time period.

For example, FIG. **60** illustrates a method **900** for performing an assay for biological or chemical analysis. In particular embodiments, the assay may include a sequencing-by-synthesis (SBS) protocol. The method **900** includes flowing reagents through a flow channel of a flow cell at **902**. The flow cell may have a sample area that includes a sample with biomolecules configured to chemically react with the reagents. The method **900** also includes illuminating the sample area at **904** with first and second semiconductor light sources (SLSs). The first and second SLSs provide first and second excitation spectra, respectively. The biomolecules of the sample may provide light emissions that are indicative of a binding reaction when illuminated by the first or second SLSs. Furthermore, the method **900** includes detecting the light emissions from the sample area at **906**. Optionally, the method **900** may include moving the flow cell at **908** relative to an imaging lens and repeating the illuminating and detecting operations **904** and **906**. The steps shown in FIG. **60** and exemplified above can be repeated for multiple cycles of a sequencing method.

FIGS. **39** and **40** illustrate various features of a motion-control system **700** formed in accordance with one embodiment that may be used with the imaging system **600**. The motion-control system **700** includes an optical base plate **702** and a sample deck **708** that is movably coupled to the base plate **702**. As shown, the base plate **702** has a support side **704** and a bottom side **705**. The support and bottom sides **704** and **705** face in opposite directions along the Z-axis. The base plate **702** is configured to support a majority of the optical components of the optical assembly **602** (FIG. **38**) on the support side **704**. The base plate **702** and the sample deck **708** may be movably coupled to each other by an intermediate support **715** and a face plate **722** such that the sample holder **650** may substantially rotate about the X and Y axes, shift along the Y axis, and slide along the X axis.

FIG. **40** is an isolated perspective view of the intermediate support **715**, a motor assembly **724**, and a movable platform **726** of the sample deck **708** (FIG. **39**). The motor assembly **724** is operatively coupled to the platform **726** and is configured to slide the platform **726** bi-directionally along the X-axis. As shown, the intermediate support **715** includes a tail end **728** and an imaging end **730**. The intermediate support **715** may include pins **746** and **748** proximate to the imaging end **730** that project away from each other along the Y-axis. Proximate to the imaging end **730**, the intermediate support **715** may include a lens opening **750** that is sized and shaped to allow the imaging lens **623** (FIG. **38**) to extend therethrough. In the illustrated embodiment, the pins **746** and **748** have a common line **755** extending therethrough that also extends through the lens opening **750**.

Returning to FIG. **39**, the platform **726** is coupled to the bottom side **705** through the intermediate support **715**. Accordingly, a weight of the sample deck **708** may be supported by the base plate **702**. Furthermore, the motion-control system **700** may include a plurality of alignment devices **733**, **735**, **737**, and **739** that are configured to position the sample holder **650**. In the illustrated embodiment, the alignment devices **733**, **735**, **737**, and **739** are micrometers. The alignment device **733** is operatively coupled to the tail end **728** of the intermediate support **715**. When the alignment device **733** is activated, the tail end **728** may be moved in a direction along the Z-axis. Consequently, the intermediate support **715** may rotate about the pins **746**

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and **748** (FIG. **40**) or, more specifically, about the line **755**. When the alignment devices **735** and **737** are activated, the sample holder **650** may shift along the Y-axis as directed. When the alignment device **739** is activated, the sample holder **650** may rotate about an axis of rotation  $R_7$  that extends parallel to the X-axis.

FIGS. **41-42** show a perspective view and plan view, respectively, of the optical base plate **702** that may be used with the imaging system **600** (FIG. **38**). In some embodiments of the imaging system **600**, one or more of the optical components **621-627**, **631-636**, and **641-645** (FIG. **38**) can have a fixed position in the optical assembly **602** such that the fixed (or static) optical component does not move during operation of the imaging system **600**. For example, the base plate **702** is configured to support a plurality of optical components and other parts of the imaging system **600**. As shown, the base plate **702** constitutes a substantially unitary structure having a support side (or surface) **704** that faces in a direction along the Z-axis. In the illustrated embodiment, the support side **704** is not continuously smooth, but may have various platforms **716-718**, depressions (or receiving spaces) **719-721**, and component-receiving spaces **711-714** that are located to arrange the optical assembly **602** in a predetermined configuration. As shown in FIG. **42**, each of the component-receiving spaces **711-714** has respective reference surfaces **781-784**. In some embodiments, the reference surfaces **781-784** can facilitate orienting and holding corresponding optical components in desired positions.

FIGS. **43** and **44** show a front perspective view and a cutaway rear perspective view, respectively, of an optical device **732**. As shown in FIG. **43**, the optical device **732** is oriented relative to mutually perpendicular axes **791-793**. The axis **791** may extend along a gravitational force direction and/or parallel to the Z-axis illustrated above. In particular embodiments, the optical device **732** is configured to be positioned within the component-receiving space **713** (FIG. **43**) of the base plate **702** (only a portion of the base plate **702** is shown in FIGS. **43** and **44**).

The component-receiving space **713** has one or more surfaces that define an accessible spatial region where an optical component may be held. These one or more surfaces may include the reference surface(s) described below. In the illustrated embodiment, the component-receiving space **713** is a component cavity of the base plate **701** that extends a depth within the base plate **702**. However, the base plate **702** may form the component-receiving space in other manners. For example, in a similar way that the base plate **702** may form a cavity, the base plate **702** may also have one or more raised platforms including surfaces that surround and define the component-receiving space. Accordingly, the base plate **702** may be shaped to partially or exclusively provide the component-receiving space. The base plate **702** may include the reference surface. In alternative embodiments, sidewalls may be mounted on the base plate **702** and configured to define the spatial region. Furthermore, other optical devices mounted to the base plate **702** may define the component-receiving spaces. As used herein, when an element "defines" a component-receiving space, the element may exclusively define the component-receiving space or may only partially define the component-receiving space.

The optical device **732** can be removably mounted to the base plate **702** in the component-receiving space **713**, but may be configured to remain in a fixed position during operation of the imaging system. However, in alternative embodiment, the optical device **732** is not positioned within the component-receiving space **713**, but may be positioned elsewhere, such as on a platform of the support side **704**. In



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the illustrated embodiment, the optical device **732** includes a mounting device **734** and an optical component **736** that is configured to reflect and/or transmit light therethrough. The mounting device **734** is configured to facilitate holding the optical component **736** in a desired orientation and also removably mount the optical component **736** to the base plate **702**. The mounting device **734** includes a component retainer **738** and a biasing element **740** that is operatively coupled to the retainer **738**.

In the illustrated embodiment, the optical component **736** comprises an optical filter that transmits optical signals therethrough while filtering for a predetermined spectrum. However, other optical components may be used in alternative embodiments, such as lenses or mirrors. As shown, the optical component **736** may include optical surfaces **742** and **744** that face in opposite directions and define a thickness  $T_3$  of the optical component **736** therebetween. As shown, the optical surfaces **742** and **744** may be continuously smooth and planar surfaces that extend parallel to each other such that the thickness  $T_3$  is substantially uniform. However, the optical surfaces **742** and **744** may have other contours in alternative embodiments. The optical component **736** may have a plurality of component edges **751-754** (FIG. **43**) that define a perimeter or periphery. The periphery surrounds the optical surfaces **742** and **744**. As shown, the periphery is substantially rectangular, but other geometries may be used in alternative embodiments (e.g., circular).

The retainer **738** facilitates holding the optical component **736** in a desired orientation. In the illustrated embodiment, the retainer **738** is configured to engage the optical surface **742** and extend around at least a portion of the periphery to retain the optical component **736**. For example, the retainer **738** may include a wall portion **756** and a frame extension **758** that extends from the wall portion **756** along the periphery of the optical component **736** (e.g., the component edge **752** (FIG. **43**)). In the illustrated embodiment, the frame extension **758** may form a bracket that limits movement of the optical component **736**. More specifically, the frame extension **758** may include a proximal arm **760** and a distal arm **762**. The proximal arm **760** extends from the wall portion **756** along the component edge **752** and the axis **791**. The distal arm **762** extends from the proximal arm **760** along the component edge **751**. The distal arm **762** includes a projection or feature **764** that extends toward and engages the optical component **736**. Also shown, the retainer **738** may include a grip member **766** that is located opposite the frame extension **758**. The grip member **766** and the frame extension **758** may cooperate in limiting movement of the optical component **736** along the axis **793**. The retainer **738** may grip a portion of the periphery of the optical component **736**.

As shown in FIGS. **43** and **44**, the wall portion **756** is configured to engage the optical surface **742**. For example, the wall portion **756** has a mating surface **770** (FIG. **43**) that faces the optical component **736**. In some embodiments, the wall portion **756** includes a plurality of orientation features **771-773** (FIG. **43**) along the mating surface **770**. The orientation features **771-773** are configured to directly engage the optical surface **742** of the optical component **736**. When the orientation features **771-773** directly engage the optical surface **742**, the optical surface **742** (and consequently the optical component **736**) is positioned in a desired orientation with respect to the retainer **738**. As shown in FIG. **43**, the reference surface **783** of the component-receiving space **713** also includes a plurality of orientation features **761-763**. The orientation features **761-763** are configured to directly engage the optical surface **744**. Further-

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more, the orientation features **761-763** may be arranged such that each of the orientation features **761-763** generally opposes a corresponding one of the orientation features **771-773**.

Also shown in FIG. **44**, the wall portion **756** has a non-mating surface **774** that faces in an opposite direction with respect to the mating surface **770** (FIG. **43**). The wall portion **756** includes an element projection **776** that extends away from the non-mating surface **774** and the optical component **736**. The biasing element **740** is configured to couple to the element projection **776**. In the illustrated embodiment, the element projection **776** and the biasing element **740** extend into a slot **778** of the component-receiving space **713**. The slot **778** is sized and shaped to receive the biasing element **740**. The slot **778** has an element surface **780** that engages the biasing element **740**.

FIG. **45** shows an isolated front view of the optical device **732**, and FIG. **46** shows how the optical device **732** may be removably mounted to the base plate **702**. To removably mount the optical component **736**, the optical component **736** may be positioned within a component-receiving space **789** of the mounting device **734** that is generally defined by the wall portion **756** (FIG. **46**), the frame extension **758**, and the grip member **766**. In particular embodiments, when the optical component **736** is positioned within the mounting device **734**, the optical component **736** is freely held within the component-receiving space **789**. For instance, the optical component **736** may not form an interference fit with the retainer **738**. Instead, during a mounting operation, the optical component **736** may be held within the component-receiving space **789** by the wall portion **756**, the frame extension **758**, the grip member **766** and, for example, an individual's hand. However, in alternative embodiments, the optical component **736** may form an interference fit with the retainer **738** or may be confined within a space that is defined only by the retainer **738**.

With respect to FIG. **46**, during the mounting operation, the biasing element **740** may be initially compressed so that the mounting device **734** may clear and be inserted into the component-receiving space **713**. For example, the biasing element **740** may be compressed by an individual's finger to reduce the size of the optical device **732**, or the biasing element **740** may be compressed by first pressing the biasing element **740** against the element surface **780** and then advancing the retainer **738** into the component-receiving space **713**. Once the optical device **732** is placed within the component-receiving space **713**, the stored mechanical energy of the compressed biasing element **740** may move the retainer **738** and the optical component **736** toward the reference surface **783** until the optical surface **744** directly engages the reference surface **783**. More specifically, the optical surface **744** may directly engage the orientation features **761-763** (FIG. **43**) of the reference surface **783**. As shown in FIG. **46**, when the optical component **736** is mounted, a small gap  $G_1$  may exist between the optical surface **742** and the mating surface **770** (FIG. **43**) because of the orientation features **771-773** (FIG. **43**), and a small gap  $G_2$  may exist between the optical surface **744** and the reference surface **783** because of the orientation features **761-763** (FIG. **43**).

In the mounted position, the biasing element **740** provides an alignment force  $F_A$  that holds the optical surface **744** against the reference surface **783**. The optical and reference surfaces **744** and **783** may be configured to position the optical component **736** in a predetermined orientation. The alignment force  $F_A$  is sufficient to hold the optical component **736** in the predetermined orientation throughout opera-



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tion of the imaging system. In other words, the mounting device 734 and the reference surface 783 may prevent the optical component 736 from moving in a direction along the axis 792. Furthermore, in the mounted position, the projection 764 (FIG. 43) may press against the component edge 751 (FIG. 43) to prevent the optical component 736 from moving in a direction along the axis 791. The frame extension 758 and the grip member 766 may prevent or limit movement of the optical component 736 in a direction along the axis 793. Accordingly, the component-receiving space 713 and the mounting device 734 may be configured with respect to each other to hold the optical component 736 in a predetermined orientation during imaging sessions.

As shown in FIG. 45, when the optical component 736 is in the mounted position, a space portion 798 of the optical surface 744 may face and interface with the reference surface 783, and a path portion 799 of the optical surface 744 may extend beyond the support side 704 into an optical path taken by optical signals. Also shown in FIG. 46, the component-receiving space 713 may extend a depth  $D_c$  into the base plate 702 from the support side 704.

The biasing element 740 may comprise any elastic member capable of storing mechanical energy to provide the alignment force  $F_A$ . In the illustrated embodiment, the elastic member comprises a coil spring that pushes the optical surface 744 against the reference surface 783 when compressed. However, in alternative embodiments, the elastic member and the component-receiving space may be configured such that the elastic member pulls the optical surface against the reference surface when extended. For example, a coil spring may have opposite ends in which one end is attached to the element surface in a slot that extends from the reference surface and another end is attached to the retainer. When the coil spring is extended, the coil spring may provide an alignment force that pulls the optical component against the reference surface. In this alternative embodiment, a rubber band may also be used.

In alternative embodiments, the mounting device 734 may be used to affix the optical component 736 to the base plate 702 using an adhesive. More specifically, the optical component 736 may be held against the reference surface 783 by the mounting device 734. An adhesive may be deposited into the gap  $G_2$  between the optical surface 744 and the reference surface 783. After the adhesive cures, the mounting device 734 may be removed while the optical component 736 remains affixed to the reference surface 783 by the adhesive.

FIG. 47 is a block diagram illustrating a method 800 of assembling an optical train. The method 800 includes providing an optical base plate at 802 that has a component-receiving space. The base plate and the component-receiving space may be similar to the base plate 702 and the component-receiving space 713 described above. The method 800 also includes inserting an optical component at 804 into the component-receiving space. The optical component may be similar to the optical component 736 described above and include an optical surface that is configured to reflect or transmit light therethrough. The optical surface may have a space portion that faces a reference surface of the component-receiving space and a path portion that extends beyond the support side into an optical path. The method 800 also includes providing an alignment force at 806 that holds the optical surface against the reference surface to orient the optical component. The optical and reference surfaces may be configured to hold the optical component in a predetermined orientation when the alignment force is provided. In some embodiments, the method 800 may also include removing the optical compo-

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nent at 808 and, optionally, inserting a different optical component at 810 into the component-receiving space. The different optical component may have the same or different optical qualities. In other words, the different optical component may be a replacement that has the same optical qualities or the different optical component may have different optical qualities.

FIGS. 48 and 49 provide a perspective view and a side view, respectively, of the light source (or excitation light module) 604. As used herein, a light source module includes one or more light sources (e.g., lasers, arc lamps, LEDs, laser diodes) that are secured to a module frame and also includes one or more optical components (e.g., lenses or filters) that are secured to the module frame in a fixed and predetermined position with respect to said one or more light sources. The light source modules may be configured to be removably coupled within an imaging system so that a user may relatively quickly install or replace the light source module. In particular embodiments, the light source module 604 constitutes a SLS module 604 that includes the first and second SLSs 614 and 616. As shown, the SLS module 604 includes a module frame 660 and a module cover 662. A plurality of imaging components may be secured to the module frame 660 in fixed positions with respect to each other. For example, the first and second SLSs 614 and 616, the excitation filter 635, and the lenses 624 and 625 may be mounted onto the module frame 660. In addition, the SLS module 604 may include first and second heat sinks 664 (FIG. 48) and 666 that are configured to transfer thermal energy from the first and second SLSs 614 and 616, respectively.

The SLS module 604 and the module frame 660 may be sized and shaped such that an individual could hold the SLS module 604 with the individual's hands and readily manipulate for installing into the imaging system 600. As such, the SLS module 604 has a weight that an adult individual could support.

The SLS module 604 is configured to be placed within the module-receiving space 719 (FIG. 41) and removably coupled to the base plate 702 (FIG. 41). As shown, the module frame 660 has a plurality of sides including a mounting side 670 and an engagement face 671 (FIG. 48). In the illustrated embodiment, the module frame 660 is substantially rectangular or block-shaped, but the module frame 660 may have other shapes in alternative embodiments. The mounting side 670 is configured to be mounted to the base plate 702 within the module-receiving space 719. As such, at least a portion of the module-receiving space 719 may be shaped to receive and hold the SLS module 604. Similar to the component-receiving space 713, the module-receiving space 719 may be defined by one or more surfaces that provide an accessible spatial region where the SLS module 604 may be held. The surface(s) may be of the base plate 702. For example, in the illustrated embodiment, the module-receiving space 719 is a depression of the base plate 702. The mounting side 670 may have a contour that substantially complements the base plate 702 and, more specifically, the module-receiving space 719. For example, the mounting side 670 may be substantially planar and include a guidance pin 672 (FIG. 49) projecting therefrom that is configured to be inserted into a corresponding hole (not shown) in the base plate 702. The guidance pin 672 may be a fastener (e.g., screw) configured to facilitate removably coupling the module frame 660 to the base plate 702. In particular embodiments, the guidance pin 672 is inserted into the base plate 702 at a non-orthogonal angle. As shown in FIG. 49, the heat sink 666 may be coupled to the module



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frame **660** such that an offset **676** exists from the mounting side **670** to the heat sink **666**.

The module frame **660** may include first and second light passages **682** and **684** that intersect each other at a passage intersection **685**. The SLSs **614** and **616** may be secured to the module frame **660** and have fixed positions with respect to each other. The SLSs **614** and **616** are oriented such that optical signals are substantially directed along optical paths through the respective light passages **682** and **684** toward the passage intersection **685**. The optical paths may be directed toward the excitation filter **635**. In the illustrated embodiment, the optical paths are perpendicular to one another until reaching the excitation filter **635**. The excitation filter **635** is oriented to reflect at least a portion of the optical signals generated by the SLS **616** and transmit at least a portion of the optical signals generated by the SLS **614**. As shown, the optical signals from each of the SLSs **614** and **616** are directed along a common path and exit the SLS module **604** through a common module window **674**. The module window **674** extends through the engagement face **671**.

FIG. **50** is a plan view of the SLS module **604** mounted onto the base plate **702**. In the illustrated embodiment, the SLS module **604** is configured to rest on the base plate **702** such that the gravitational force  $g$  facilitates holding the SLS module **604** thereon. As such, the SLS module **604** may provide an integrated device that is readily removed or separated from the optical assembly **600**. For example, after removing a housing (not shown) of the assay system or after receiving access to the optical assembly, the SLS module **604** may be grabbed by an individual and removed or replaced. When the SLS module **604** is located on the base plate **702**, the engagement face **671** may engage an optical device **680**. The optical device **680** may be adjacent to the module window **674** such that the optical signals generated by the SLS module **604** are transmitted through the optical device **680**.

Although the illustrated embodiment is described as using an SLS module with first and second SLSs, excitation light may be directed onto the sample in other manners. For example, the SLS module **604** may include only one SLS and another optical component (e.g., lens or filter) having fixed positions with respect to each other in a module frame. Likewise, more than two SLSs may be used. In a similar manner, light modules may include only one laser or more than two lasers.

However, embodiments described herein are not limited to only having modular excitation systems, such as the SLS module **604**. For example, the imaging system **600** may use a light source that is not mounted to a module frame. More specifically, a laser could be directly mounted to the base plate or other portion of the imaging system or may be mounted to a frame that, in turn, is mounted within the imaging system.

Returning to FIG. **38**, the imaging system **600** may have an image-focusing system **840** that includes the object or sample holder **650**, an optical train **842**, and the imaging detector **610**. The optical train **842** is configured to direct optical signals from the sample holder **650** (e.g., light emissions from the sample area **608** of the flow cell **606**) to a detector surface **844** of the imaging detector **610**. As shown in FIG. **38**, the optical train **842** includes the optical components **623**, **644**, **634**, **633**, **621**, **631**, and **642**. The optical train **842** may include other optical components. In the illustrated configuration, the optical train **842** has an object or sample plane **846** located proximate to the sample holder **650** and an image plane **848** located proximate to the

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detector surface **844**. The imaging detector **610** is configured to obtain object or sample images at the detector surface **844**.

In some embodiments, the image-focusing system **840** is configured to move the image plane **848** relative to the detector **610** and capture a test image. More specifically, the image plane **848** may be moved such that the image plane **848** extends in a non-parallel manner with respect to the detector surface **844** and intersects the detector surface **844**. A location of the intersection may be determined by analyzing the test image. The location may then be used to determine a degree-of-focus of the imaging system **600**. In particular embodiments, the image-focusing system **840** utilizes a rotatable mirror that is operatively coupled to an actuator for moving the rotatable mirror. However, the image-focusing system **840** may move other optical components that direct the optical signals to the detector surface **844**, or the image-focusing system **840** may move the detector **610**. In either case, the image plane **848** may be relatively moved with respect to the detector surface **844**. For example, the image-focusing system **840** may move a lens.

In particular embodiments, the imaging detector **610** is configured to obtain test images using a rotatable mirror **642** to determine a degree-of-focus of the imaging system **600**. As a result of the determined degree-of-focus, the imaging system **600** may move the sample holder **650** so that the object or sample is located within the sample plane **846**. For example, the sample holder **650** may be configured to move the sample area **608** in a  $z$ -direction a predetermined distance (as indicated by  $\Delta z$ ).

FIG. **51** is a plan view that illustrates several of the components in the image-focusing system **840**. As shown, the image-focusing system **840** includes a rotatable mirror assembly **850** that includes the mirror **642**, a mounting assembly **852** having the mirror **642** mounted thereon, and an actuator or rotation mechanism **854** that is configured to rotate the mounting assembly **852** and the mirror **642** about an axis of rotation  $R_6$ . The mirror **642** is configured to reflect optical signals **863** that are received from the sample area **608** (FIG. **38**) toward the imaging detector **610** and onto the detector surface **844**. In the illustrated embodiment, the mirror **642** reflects the optical signals **863** directly onto the detector surface **844** (i.e., there are no intervening optical components that redirect the optical signals **863**). However, in alternative embodiments, there may be additional optical components that affect the propagation of the optical signals **863**.

In the illustrated embodiment, the image-focusing system **840** also includes positive stops **860** and **862** that are configured to prevent the mirror **642** from rotating beyond predetermined rotational positions. The positive stops **860** and **862** have fixed positions with respect to the axis  $R_6$ . The mounting assembly **852** is configured to pivot about the axis  $R_6$  between the positive stops **860** and **862** depending upon whether sample images or test images are being obtained. Accordingly, the mirror **642** may be rotated between a test position (or orientation) and an imaging position (or orientation). By way of example only, the mirror **642** may be rotated from approximately  $5^\circ$  to approximately  $12^\circ$  about the axis  $R_6$  between the different rotational positions. In particular embodiments, the mirror **642** may be rotated approximately  $8^\circ$  about the axis  $R_6$ .

FIG. **52** is a perspective view of the mirror assembly **850**. As shown, the mounting assembly **852** includes an interior frame **864** and a support bracket **866**. The interior frame **864** is configured to couple to the mirror **642** and also to the



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support bracket **866**. The interior frame **864** and the support bracket **866** may interact with each other and a plurality of set screws **868** to provide minor adjustments to the orientation of the mirror **642**. As such, the mounting assembly **852** may constitute a gimbal mirror mount assembly. Also shown, the mounting assembly **852** is coupled to the rotation mechanism **854**. In the illustrated embodiment, the rotation mechanism **854** comprises a direct drive motor. However, a variety of alternative rotation mechanisms may be used, such as direct current (DC) motors, solenoid drivers, linear actuators, piezoelectric motors, and the like. Also shown in FIG. **52**, the positive stop **860** may have a fixed position with respect to the rotation mechanism **854** and the axis  $R_6$ .

As discussed above, the rotation mechanism **854** is configured to rotate or pivot the mirror **642** about the axis  $R_6$ . As shown in FIG. **52**, the mirror **642** has a geometric center C that extends along the axis  $R_6$ . The geometric center C of the mirror **642** is offset with respect to the axis  $R_6$ . In some embodiments, the rotation mechanism **854** is configured to move the mirror **642** between the test position and imaging position in less than 500 milliseconds. In particular embodiments, the rotation mechanism **854** is configured to move the mirror **642** between the test position and imaging position in less than 250 milliseconds or less than 160 milliseconds.

FIG. **53** is a schematic diagram of the mirror **642** in the imaging position. As shown, the optical signals **863** from the sample area **608** (FIG. **38**) are reflected by the mirror **642** and directed toward the detector surface **844** of the imaging detector **610**. Depending upon the configuration of the optical train **842** and the z-position of the sample holder **610**, the sample area **608** may be sufficiently in-focus or not sufficiently in-focus (i.e., out-of-focus). FIG. **53** illustrates two image planes **848A** and **848B**. The image plane **848A** substantially coincides with the detector surface **844** and, as such, the corresponding sample image has an acceptable or sufficient degree-of-focus. However, the image plane **848B** is spaced apart from the detector surface **844**. Accordingly, the sample image obtained when the image plane **848B** is spaced apart from the detector surface **844** may not have a sufficient degree-of-focus.

FIGS. **54** and **55** illustrate sample images **870** and **872**, respectively. The sample image **870** is the image detected by the imaging detector **610** when the image plane **848A** coincides with the detector surface **844**. The sample image **872** is the image detected by the imaging detector **610** when the image plane **848B** does not coincide with the detector surface **844**. (The sample images **870** and **872** include clusters of DNA that provide fluorescent light emissions when excited by predetermined excitation spectra.) As shown in FIGS. **54** and **55**, the sample image **870** has an acceptable degree-of-focus in which each of the clusters along the sample image **870** is clearly defined, and the sample image **872** does not have an acceptable degree-of-focus in which each of the clusters is clearly defined.

FIG. **56** is a schematic diagram of the mirror **642** in the focusing position. As shown, the mirror **642** in the focusing position has been rotated about the axis  $R_6$  an angle  $\theta$ . Again, the optical signals **863** from the sample area **608** (FIG. **38**) are reflected by the mirror **642** and directed toward the detector surface **844** of the imaging detector **610**. However, the optical train **842** in FIG. **56** is arranged so that the image plane **848** has been moved with respect to the detector surface **844**. More specifically, the image plane **848** does not extend parallel to the detector surface **844** and, instead, intersects the detector surface **844** at a plane intersection PI. While the mirror **642** is in the focusing position, the imaging system **600** may obtain a test image of the sample area **608**.

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As shown in FIG. **56**, the plane intersections PI may occur at different locations on the detector surface **844** depending upon the degree to which the sample area **608** is in-focus during an imaging session.

For example, FIGS. **57** and **58** illustrate test images **874** and **876**, respectively. The test image **874** represents the image obtained when the sample area **608** is in-focus, and the test image **876** represents the image obtained when the optical train **842** is out-of-focus. As shown, the test image **874** has a focused region or location  $FL_1$  that is located a distance  $XD_1$  away from a reference edge **880**, and the test image **876** has a focused region or location  $FL_2$  that is located a distance  $XD_2$  away from a reference edge **880**. The focused locations  $FL_1$  and  $FL_2$  may be determined by an image analysis module **656** (FIG. **38**).

To identify the focused locations  $FL_1$  and  $FL_2$  in the test images **874** and **876**, the image analysis module **656** may determine the location of an optimal degree-of-focus in the corresponding test image. More specifically, the analysis module **656** may determine a focus score for different points along the x-dimension of the test images **874** and **876**. The analysis module **656** may calculate the focus score at each point based on one or more image quality parameters. Examples of image quality parameters include image contrast, spot size, image signal to noise ratio, and the mean-square-error between pixels within the image. By way of example, when calculating a focus score, the analysis module **656** may calculate a coefficient of variation in contrast within the image. The coefficient of variation in contrast represents an amount of variation between intensities of the pixels in an image or a select portion of an image. As a further example, when calculating a focus score, the analysis module **656** may calculate the size of a spot derived from the image. The spot can be represented as a Gaussian spot and size can be measured as the full width half maximum (FWHM), in which case smaller spot size is typically correlated with improved focus.

After determining the focused location FL in the test image, the analysis module **656** may then measure or determine the distance XD that the focused location FL is spaced apart or separated from the reference edge **880**. The distance XD may then be correlated to a z-position of the sample area **608** with respect to the sample plane **846**. For example, the analysis module **656** may determine that the distance  $XD_2$  shown in FIG. **58** corresponds to the sample area **608** be located a distance  $\Delta z$  from the sample plane **846**. As such, the sample holder **650** may then be moved the distance  $\Delta z$  to move the sample area **608** within the sample plane **846**. Accordingly, the focused locations FL in test images may be indicative of a position of the sample area **608** with respect to the sample plane **846**. As used herein, the phrase “being indicative of a position of the object (or sample) with respect to the object (or sample) plane” includes using the factor (e.g., the focused location) to provide a more suitable model or algorithm for determining the distance  $\Delta z$ .

FIG. **59** is a block diagram illustrating a method **890** for controlling focus of an optical imaging system. The method **890** includes providing an optical train at **892** having a rotatable mirror that is configured to direct optical signals onto a detector surface. The detector surface may be similar to the detector surface **844**. The optical train may have an object plane, such as the sample plane **846**, that is proximate to an object. The optical train may also have an image plane, such as the image plane **848**, that is proximate to the detector surface. The rotatable mirror may be rotatable between an imaging position and a focusing position.



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The method **890** also includes rotating the mirror at **894** to the focusing position and obtaining a test image of the object at **896** when the mirror is in the focusing position. The test image may have an optimal degree-of-focus at a focused location. The focused location may be indicative of a position of the object with respect to the object plane. Furthermore, the method **890** may also include moving the object at **898** toward the object plane based on the focused location.

It is to be understood that the above description is intended to be illustrative, and not restrictive. For example, the above-described embodiments (and/or aspects thereof) may be used in combination with each other. In addition, many modifications may be made to embodiments without departing from the scope of the invention in order to adapt a particular situation or material. While the specific components and processes described herein are intended to define the parameters of the various embodiments, they are by no means limiting and are exemplary embodiments. Many other embodiments will be apparent to those of skill in the art upon reviewing the above description. The scope of the invention should, therefore, be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. In the appended claims, the terms “including” and “in which” are used as the plain-English equivalents of the respective terms “comprising” and “wherein.” Moreover, in the following claims, the terms “first,” “second,” and “third,” etc. are used merely as labels, and are not intended to impose numerical requirements on their objects. Further, the limitations of the following claims are not written in means-plus-function format and are not intended to be interpreted based on 35 U.S.C. § 112, sixth paragraph, unless and until such claim limitations expressly use the phrase “means for” followed by a statement of function void of further structure.

What is claimed is:

**1.** A system comprising

an optical deck having a plurality of optical components mounted thereto;

a sample deck having a slidable platform that supports a fluidic device thereon and a thermal module to control a temperature of the fluidic device; and

a fluid storage system comprising:

an enclosure having a cavity;

a door configured to open to provide access to the cavity;

a temperature control assembly configured to regulate a temperature within the cavity;

a fluid removal assembly comprising:

an elevator mechanism including a drive motor, and

a stage assembly having a transport platform to hold an array of sipper tubes disposable at least partially within the cavity,

wherein the drive motor is to move the array of sipper tubes bidirectionally along a Z-axis,

wherein each sipper tube of the array of sipper tubes includes a distal portion that is to be inserted into a component well of a reaction component tray such that fluids stored in the reaction component tray are removeable to be delivered to the fluidic device, wherein the enclosure of the fluid storage system is separate from the optical deck and separate from the sample deck; and

a casing enclosing the optical deck and the fluid storage system therein, the door providing access through the casing to the cavity.

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**2.** The system of claim **1**, further comprising a multi-port valve to selectively flow different fluids to the fluidic device, wherein each sipper tube of the array of sipper tubes is in fluid communication with the multi-port valve to selectively flow a corresponding fluid stored in a corresponding component well of the reaction component tray to the fluidic device.

**3.** The system of claim **1**, further comprising the reaction component tray comprising a plurality of component wells to store the fluids, wherein the elevator mechanism is to move the array of sipper tubes between withdrawn and deposited levels, the distal portions of the sipper tubes of the array of sipper tubes inserted into the component wells to remove fluid therefrom when at the deposited level, and the distal portions of the sipper tubes of the array of sipper tubes completely removed from the reaction component tray at the withdrawn level.

**4.** The system of claim **3**, wherein the plurality of component wells includes one or more components selected from the group consisting of a polymerase, modified nucleotides, a cleavage mix, and an oxidizing protectant.

**5.** The system of claim **3**, wherein the reaction component tray comprises a tray cover including a plurality of openings that are aligned with corresponding component wells.

**6.** The system of claim **1**, wherein the fluid storage system comprises a guide plate, wherein the distal portions of the array of sipper tubes extend through corresponding openings of the guide plate.

**7.** The system of claim **1**, wherein the fluid storage system comprises a sensor to determine a presence of the reaction component tray in the cavity.

**8.** The system of claim **1**, wherein the fluid storage system comprises a sensor to determine a level of the stage assembly and wherein when the sensor identifies the level of the stage assembly not reaching a threshold level, the system generates an alert indicative that the reaction component tray is not ready for removal.

**9.** The system of claim **1**, wherein the fluid removal assembly further comprises a plurality of support beams that extends along the Z-axis, and wherein a guide plate is affixed to the plurality of support beams.

**10.** The system of claim **1**, wherein the temperature control assembly is coupled to a rear of the enclosure of the fluid storage system opposite the door.

**11.** The system of claim **1**, wherein the fluidic device is a flow cell.

**12.** The system of claim **1**, wherein the array of sipper tubes is in fluid communication with a system pump that is to direct a flow of fluid through the array of sipper tubes.

**13.** The system of claim **1**, wherein the plurality of optical components forms an optical assembly, and wherein the slidable platform is to slide with respect to an imaging lens of an optical assembly of the optical deck.

**14.** The system of claim **1**, wherein the slidable platform is to slide bi-directionally along an X-axis.

**15.** The system of claim **1**, wherein the sample deck is to slide bi-directionally along an X-axis.

**16.** The system of claim **1**, wherein the plurality of optical components comprises a light source assembly and two imaging detectors, the light source assembly comprising a first excitation light source and a second excitation light source.

**17.** The system of claim **1**, further comprising a touch-screen user interface.

**18.** The system of claim **1**, wherein the elevator mechanism further comprises a lead screw operatively coupled to



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the drive motor, wherein rotation of the lead screw moves the transport platform bi-directionally.

19. The system of claim 18, wherein the elevator mechanism further comprises structural supports and the lead screw extends between the structural supports.

20. The system of claim 1, further comprising a pair of opposing guide rails to receive and direct the reaction component tray to a fluid removal position in the cavity.

21. The system of claim 1, wherein the reaction component tray further comprises a protective foil covering an opening at a top of individual component wells and the array of sipper tubes is to pierce the protective foil.

22. The system of claim 1, wherein the temperature control assembly comprises a thermoelectric cooling assembly.

23. The system of claim 1, wherein the temperature control assembly projects into the cavity.

24. A system comprising:

an optical deck comprising a light source assembly and two imaging detectors, wherein the light source assembly comprises a first excitation light source and a second excitation light source;

a sample deck having a slidable platform that supports a flow cell thereon and a thermal module to control a temperature of the flow cell;

a fluid storage system comprising:

an enclosure having a cavity;

a door configured to open to provide access to the cavity; a temperature control assembly projecting into the cavity to regulate a temperature within the cavity;

a fluid removal assembly comprising:

an elevator mechanism including a drive motor and a lead screw operatively coupled to the drive motor, wherein rotation of the lead screw moves the transport platform bi-directionally,

a stage assembly having a transport platform to hold an array of sipper tubes disposable at least partially within the cavity,

a guide plate having openings through which the array of sipper tubes slide, and

a plurality of support beams affixed to the guide plate and extending parallel to the lead screw;

a multi-port valve to selectively flow different fluids to the flow cell; and

a reaction component tray within the cavity of the enclosure of the fluid storage system, wherein the reaction component tray comprises a plurality of component wells to store fluids, wherein the plurality of component wells in the reaction component tray include a polymerase, modified nucleotides, a cleavage mix, and an oxidizing protectant,

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and wherein each sipper tube of the array of sipper tubes includes a distal portion that is to be inserted into one of the component wells of the reaction component tray such that the fluids stored in the reaction component tray are removeable to be delivered to the multi-port valve to selectively flow a corresponding fluid stored in a corresponding component well of the reaction component tray to the flow cell, wherein the enclosure of the fluid storage system is separate from the optical deck and separate from the sample deck; and

a casing enclosing the optical deck and the fluid storage system therein, the door providing access through the casing to the cavity.

25. The system of claim 24, wherein the thermal module is coupled to the slidable platform.

26. A system comprising

an optical deck having a plurality of optical components mounted thereto;

a sample deck having a slidable platform that supports a fluidic device thereon and a thermal module coupled to the slidable platform to control a temperature of the fluidic device; and

a fluid storage system comprising:

an enclosure having a cavity;

a door configured to open to provide access to the cavity;

a temperature control assembly configured to regulate a temperature within the cavity;

a fluid removal assembly comprising:

an elevator mechanism including a drive motor, and a stage assembly having a transport platform to hold an array of sipper tubes disposable at least partially within the cavity,

a sensor to determine a level of the stage assembly, wherein the drive motor is to move the array of sipper tubes bidirectionally along a Z-axis,

wherein each sipper tube of the array of sipper tubes includes a distal portion that is to be inserted into a component well of a reaction component tray such that fluids stored in the reaction component tray are removeable to be delivered to the fluidic device, wherein the enclosure of the fluid storage system is separate from the optical deck and separate from the sample deck; and

a casing enclosing the optical deck and the fluid storage system therein, the door providing access through the casing to the cavity,

wherein when the sensor identifies the level of the stage assembly not reaching a threshold level, the system generates an alert indicative that the reaction component tray is not ready for removal.

\* \* \* \* \*



UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 11,117,130 B2  
APPLICATION NO. : 16/874412  
DATED : September 14, 2021  
INVENTOR(S) : Erik Williamson et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1, Line 19, delete “Feb.” and insert -- Jan. --, therefor.

Column 1, Line 20, delete “Feb.” and insert -- Jan. --, therefor.

Column 1, Line 21, delete “Feb.” and insert -- Jan. --, therefor.

Column 1, Line 23, delete “Feb.” and insert -- Jan. --, therefor.

In the Claims

Claim 24, Column 55, Line 21-23, delete “detectors, wherein the light source assembly comprises a first excitation light source and a second excitation light source;” and insert -- detectors; --, therefor.

Claim 24, Column 56, Line 4, delete “t ray” and insert -- tray --, therefor.

Signed and Sealed this  
First Day of March, 2022



Drew Hirshfeld  
*Performing the Functions and Duties of the  
Under Secretary of Commerce for Intellectual Property and  
Director of the United States Patent and Trademark Office*



# EXHIBIT 5





US011697116B2

(12) **United States Patent**  
**Williamson et al.**

(10) **Patent No.:** **US 11,697,116 B2**  
(45) **Date of Patent:** **Jul. 11, 2023**

(54) **SYSTEMS, METHODS, AND APPARATUSES  
TO IMAGE A SAMPLE FOR BIOLOGICAL  
OR CHEMICAL ANALYSIS**

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(71) Applicant: **ILLUMINA, INC.**, San Diego, CA  
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(21) Appl. No.: **17/714,129**

*Primary Examiner* — Jonathan M Hurst

(22) Filed: **Apr. 5, 2022**

(74) *Attorney, Agent, or Firm* — Marshall, Gerstein &  
Borun LLP

(65) **Prior Publication Data**

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(57) **ABSTRACT**

**Related U.S. Application Data**

(60) Continuation of application No. 16/255,546, filed on  
Jan. 23, 2019, now Pat. No. 11,559,805, which is a  
(Continued)

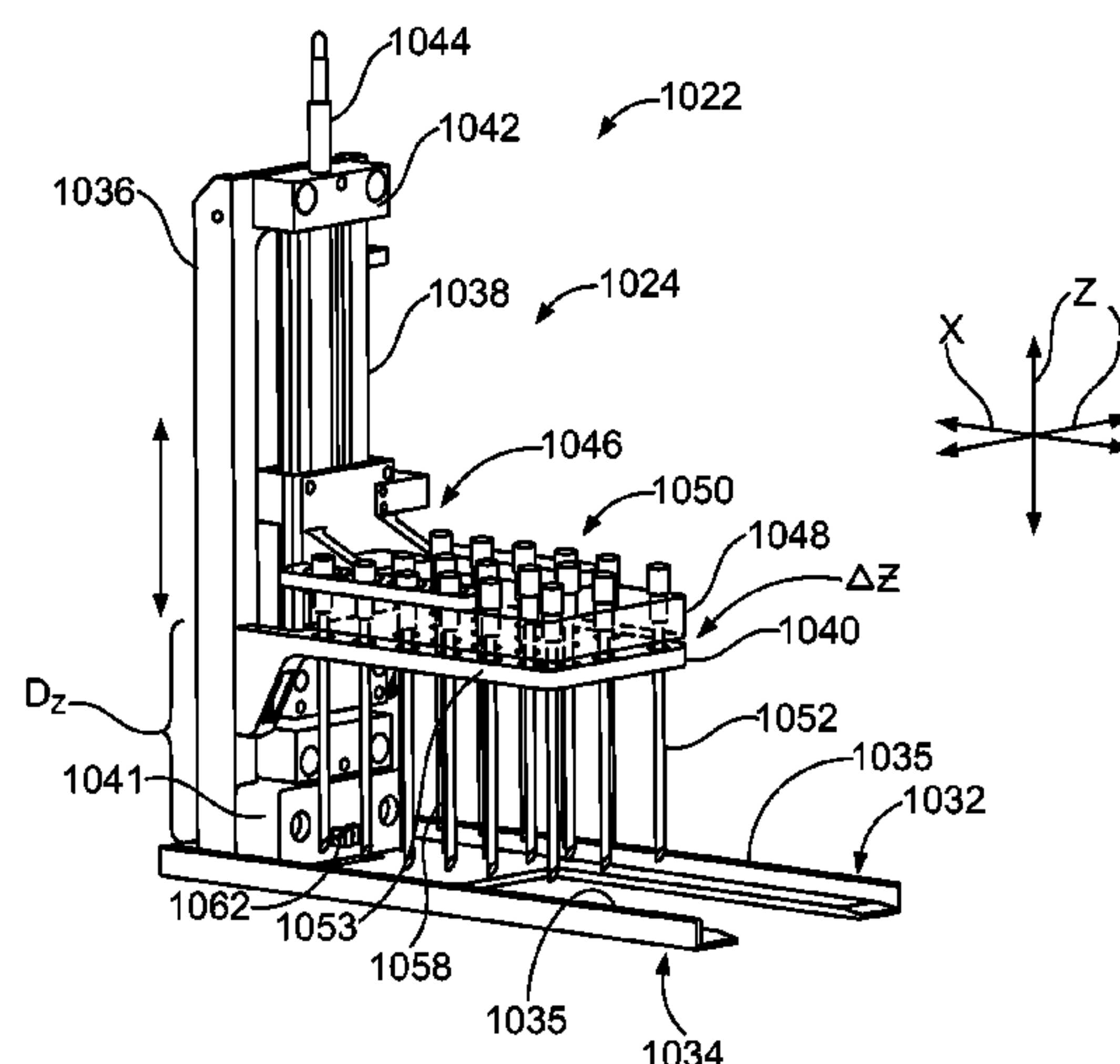
A fluidic device holder configured to orient a fluidic device.  
The device holder includes a support structure configured to  
receive a fluidic device. The support structure includes a  
base surface that faces in a direction along the Z-axis and is  
configured to have the fluidic device positioned thereon. The  
device holder also includes a plurality of reference surfaces  
facing in respective directions along an XY-plane. The  
device holder also includes an alignment assembly having  
an actuator and a movable locator arm that is operatively  
coupled to the actuator. The locator arm has an engagement  
end. The actuator moves the locator arm between retracted  
and biased positions to move the engagement end away from  
and toward the reference surfaces. The locator arm is  
configured to hold the fluidic device against the reference  
surfaces when the locator arm is in the biased position.

(51) **Int. Cl.**  
**B01L 3/00** (2006.01)  
**B01L 9/00** (2006.01)  
(Continued)

(52) **U.S. Cl.**  
CPC ..... **B01L 3/502715** (2013.01); **B01L 9/527**  
(2013.01); **G01N 21/05** (2013.01);  
(Continued)

(58) **Field of Classification Search**  
CPC ..... B01L 3/50273; B01L 2300/0816  
See application file for complete search history.

**4 Claims, 39 Drawing Sheets**





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Page 2

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(51)	Int. Cl.				
	G01N 21/05	(2006.01)			
	B01L 7/00	(2006.01)			
(52)	U.S. Cl.				
	CPC .....	B01L 3/50273 (2013.01); B01L 7/52 (2013.01); B01L 2200/025 (2013.01); B01L 2200/027 (2013.01); B01L 2200/04 (2013.01); B01L 2200/0689 (2013.01); B01L 2300/022 (2013.01); B01L 2300/041 (2013.01); B01L 2300/043 (2013.01); B01L 2300/0816 (2013.01); B01L 2300/0877 (2013.01); G01N 2021/058 (2013.01); Y10T 436/25 (2015.01)			
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	6,482,591 B2	11/2002 Lockhart et al.			
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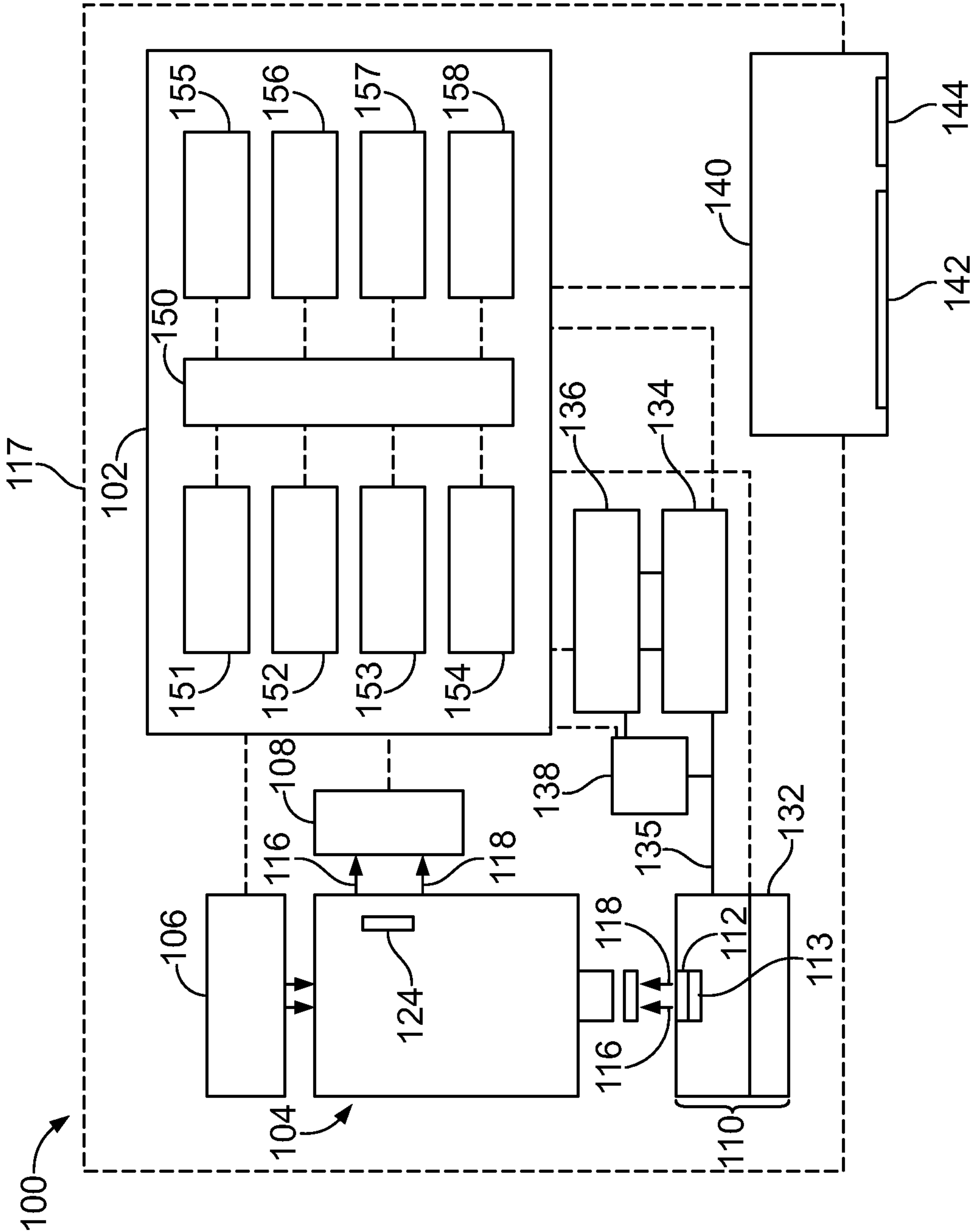


FIG. 1



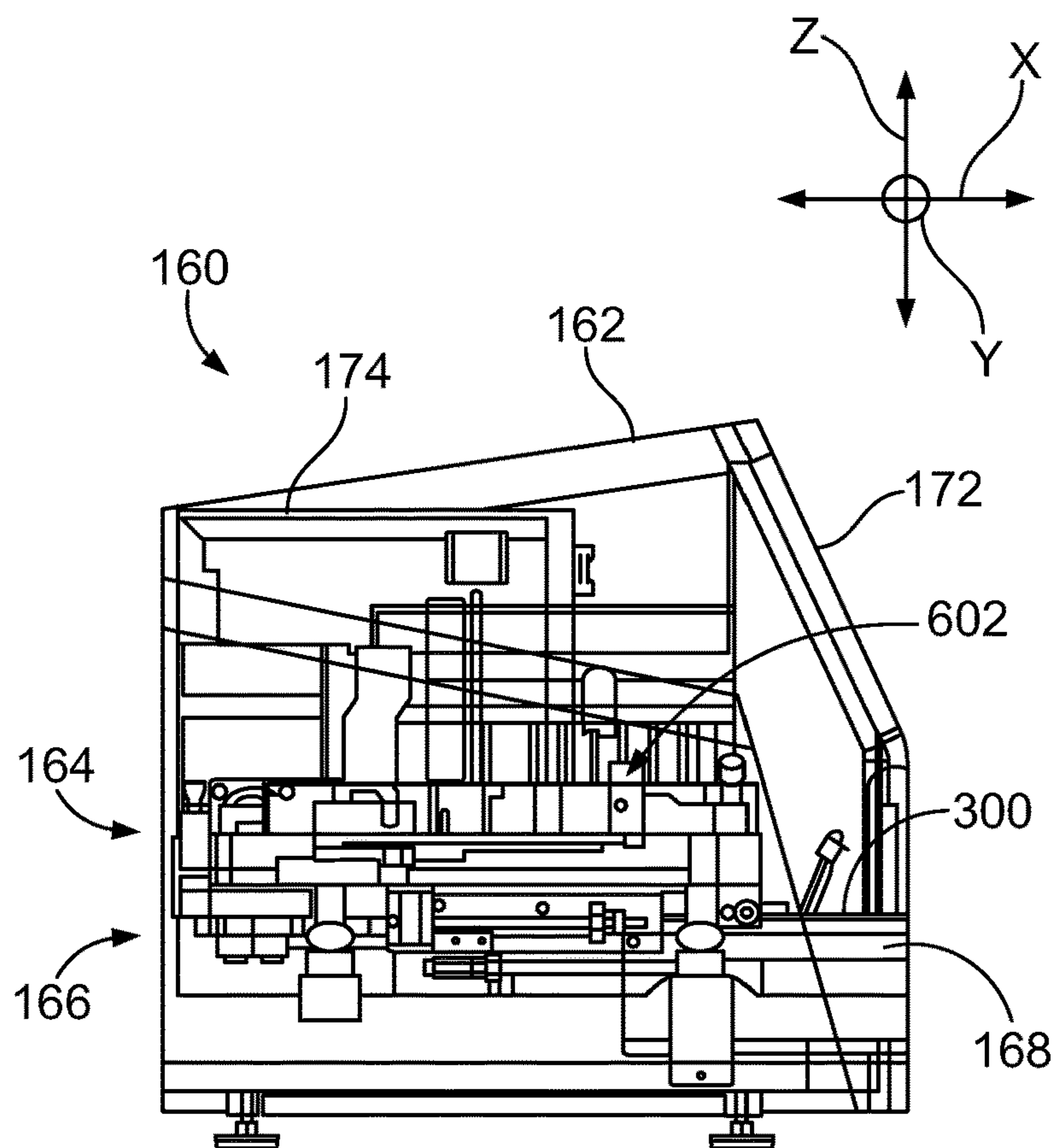


FIG. 2

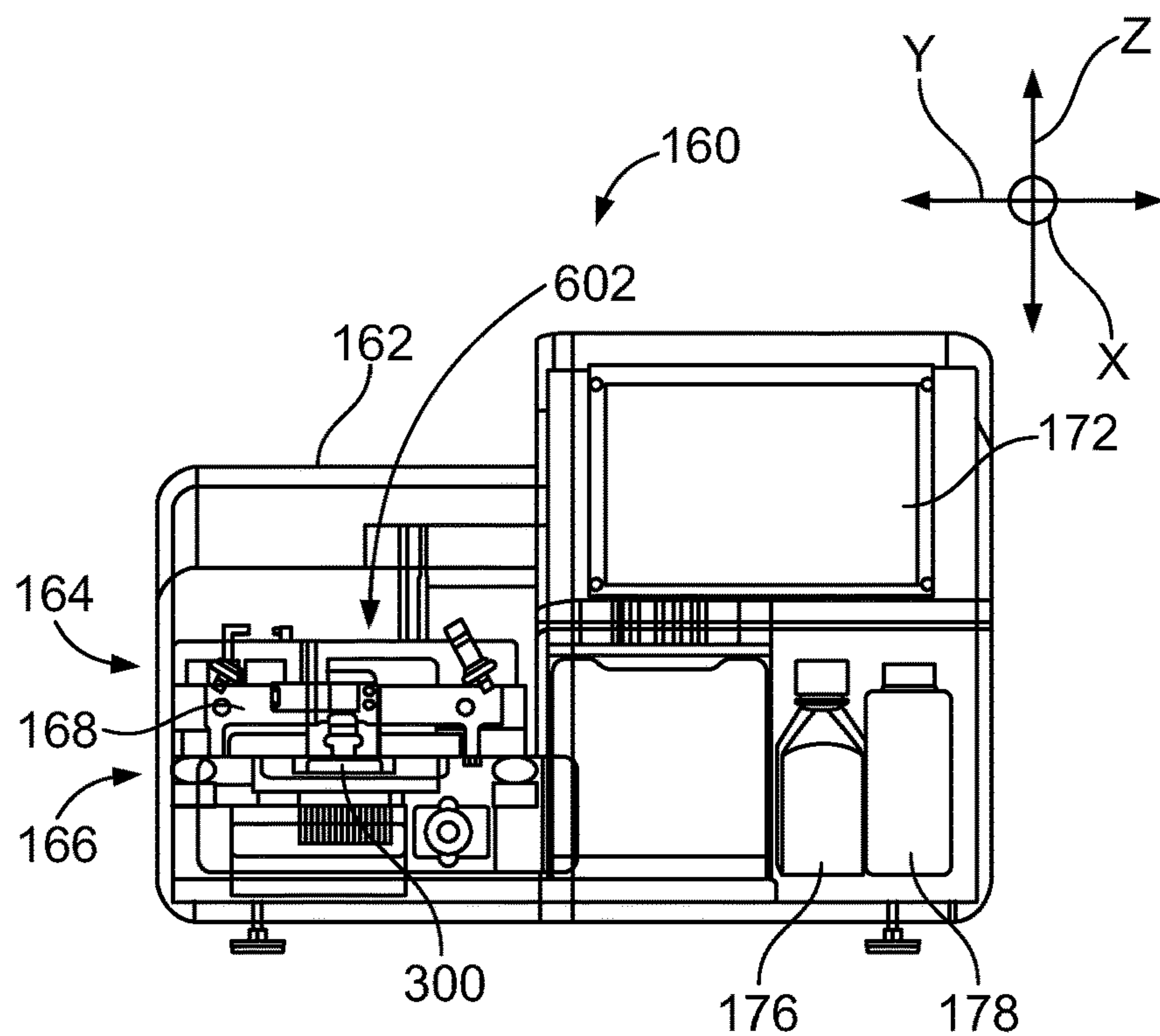


FIG. 3



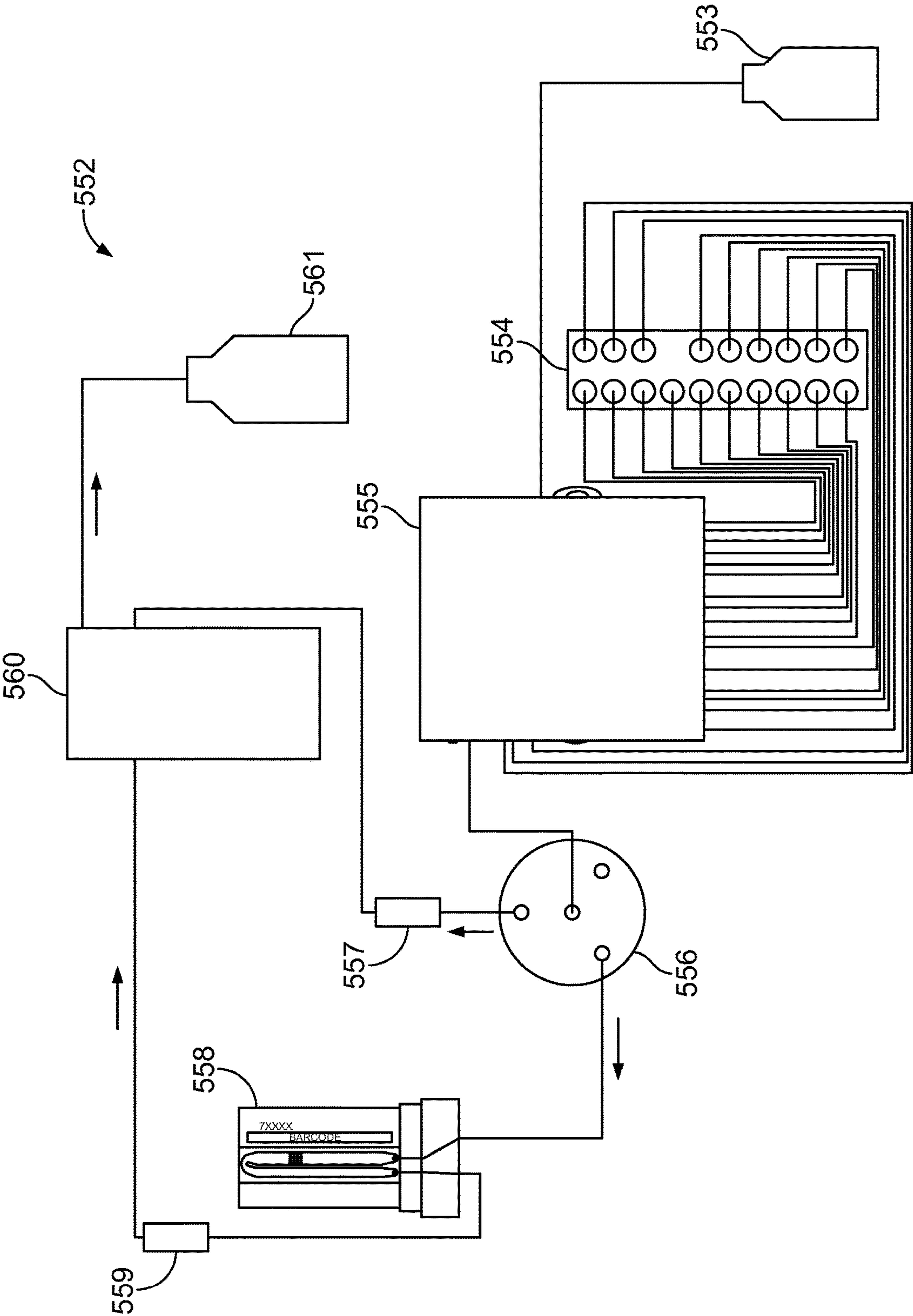


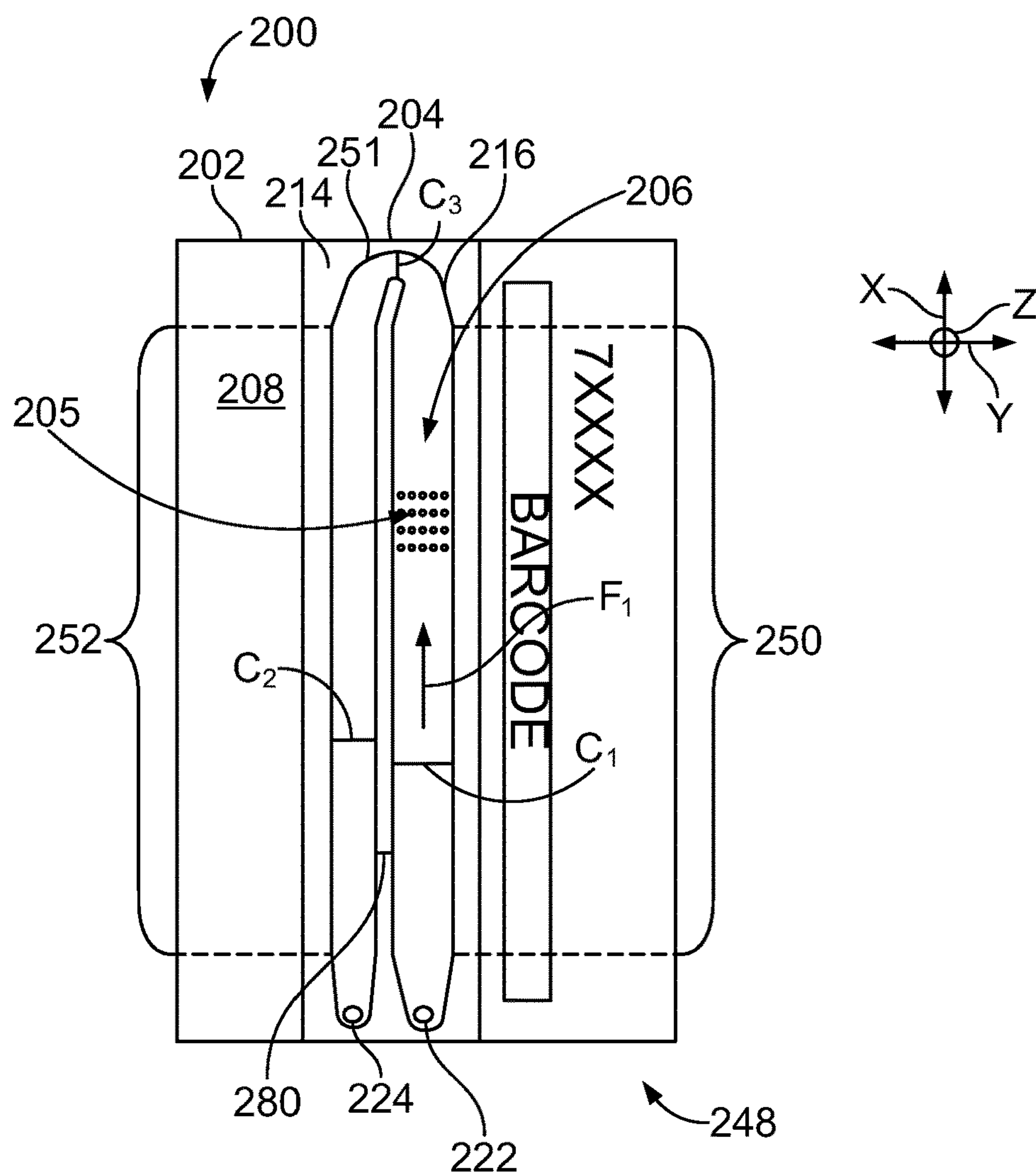
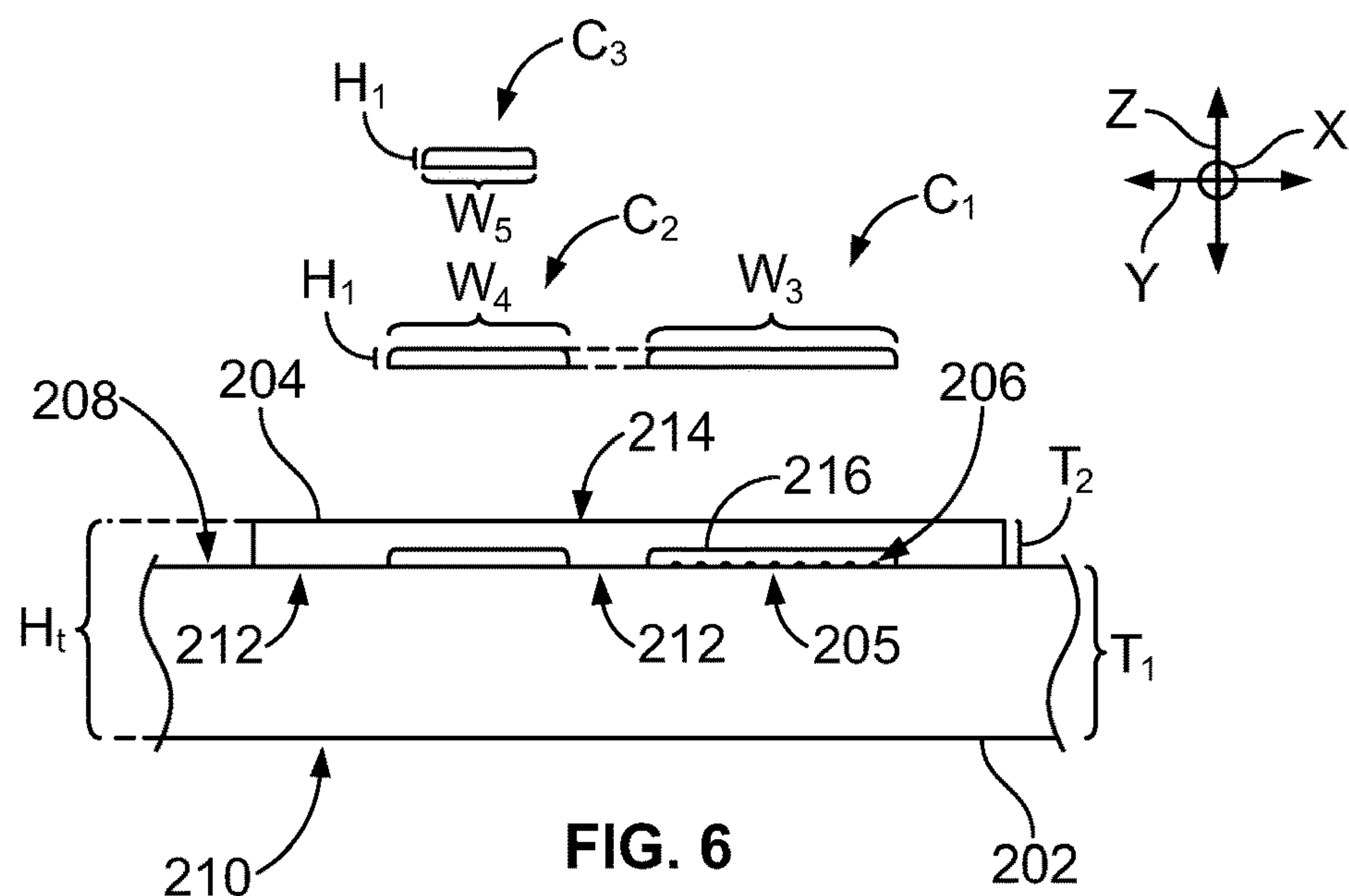
FIG. 4





FIG. 5











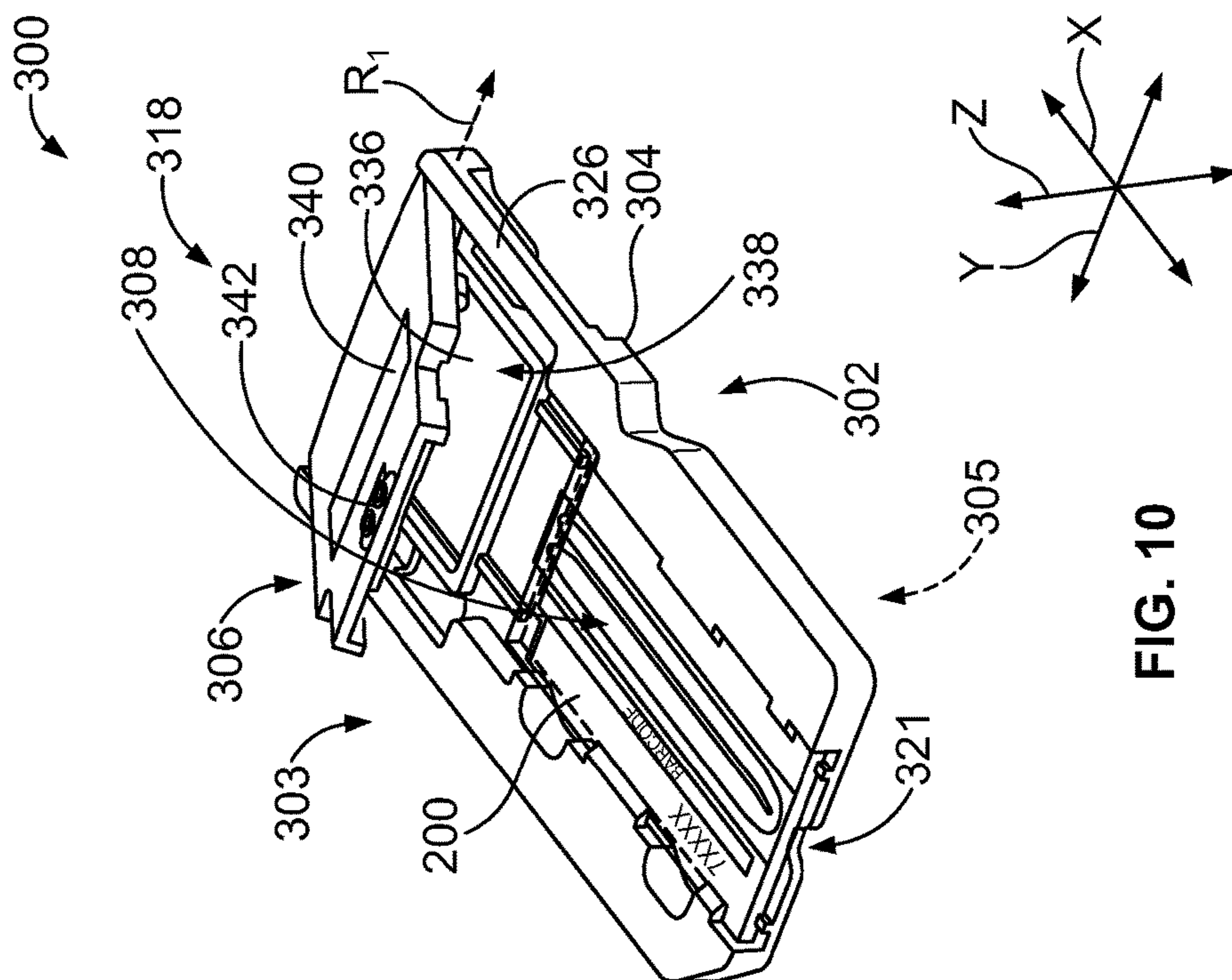


FIG. 10

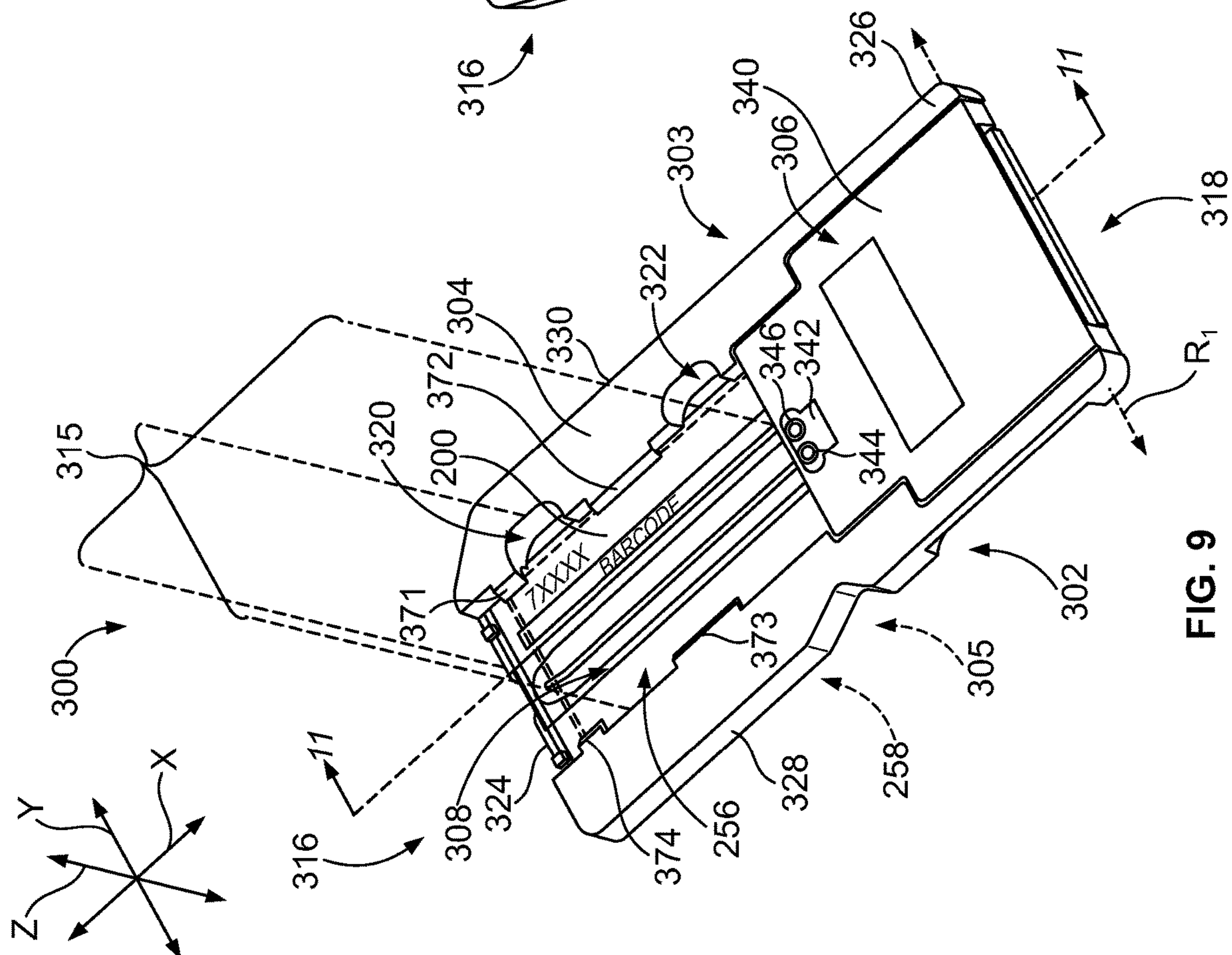
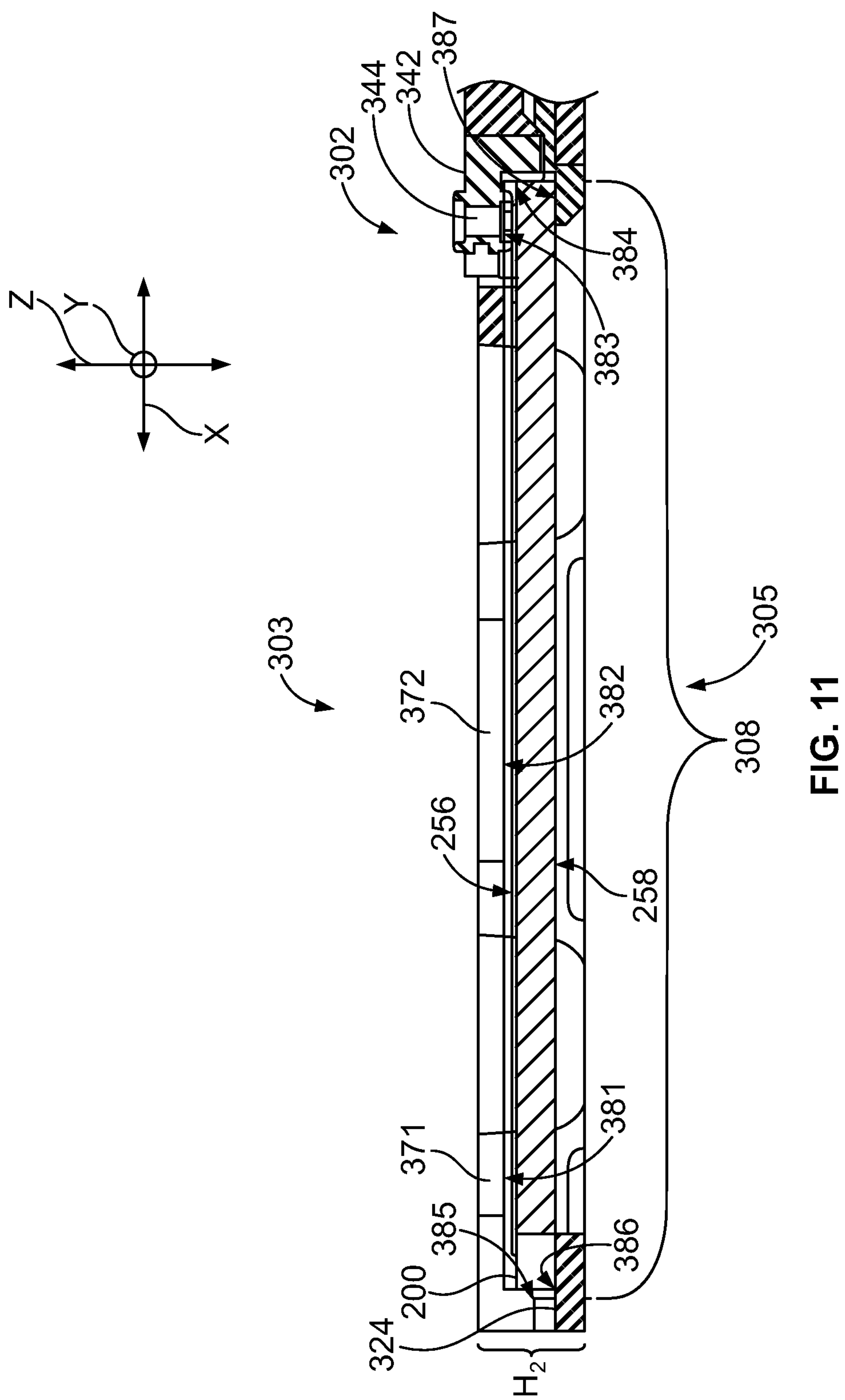


FIG. 9







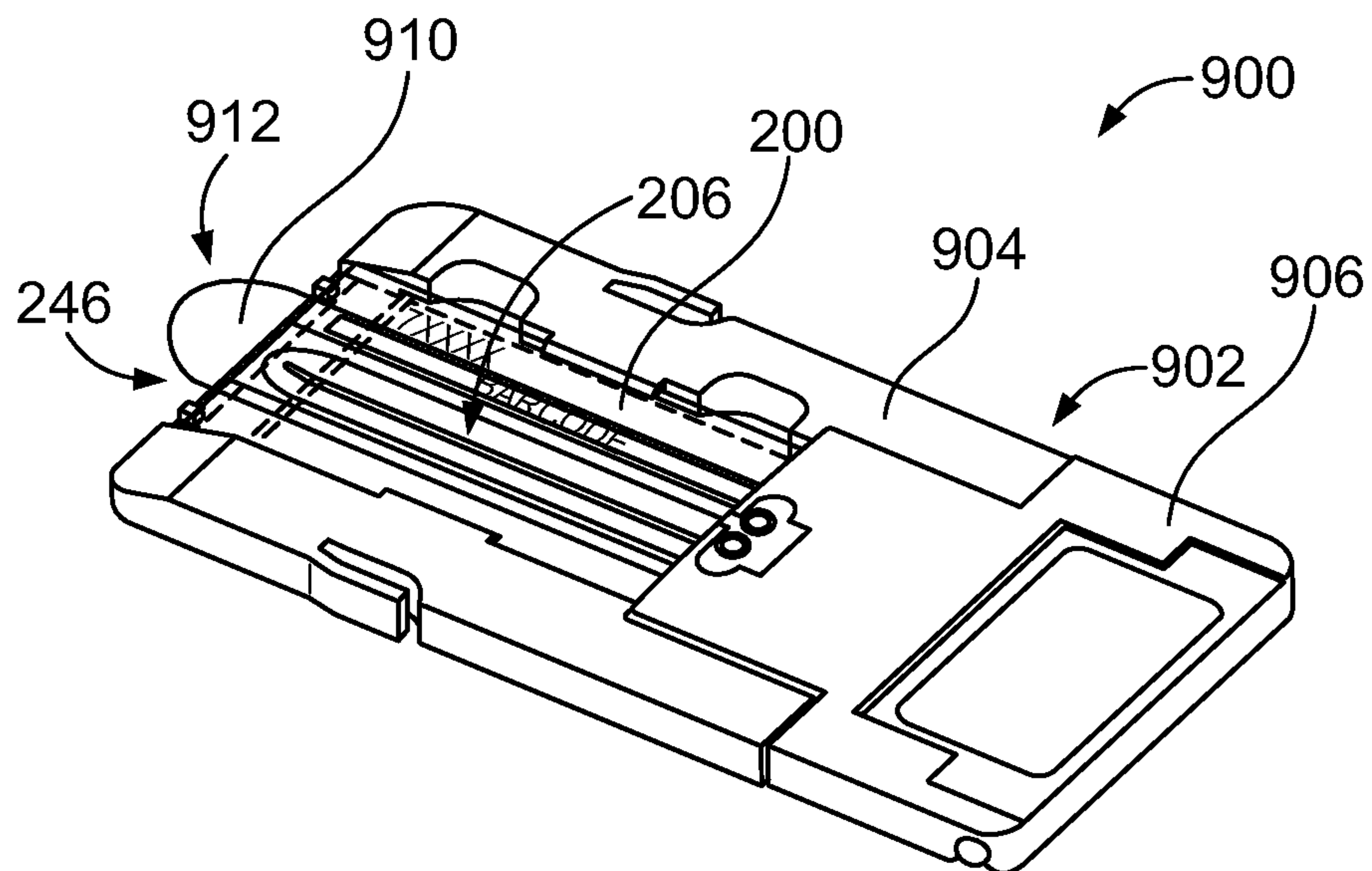


FIG. 12

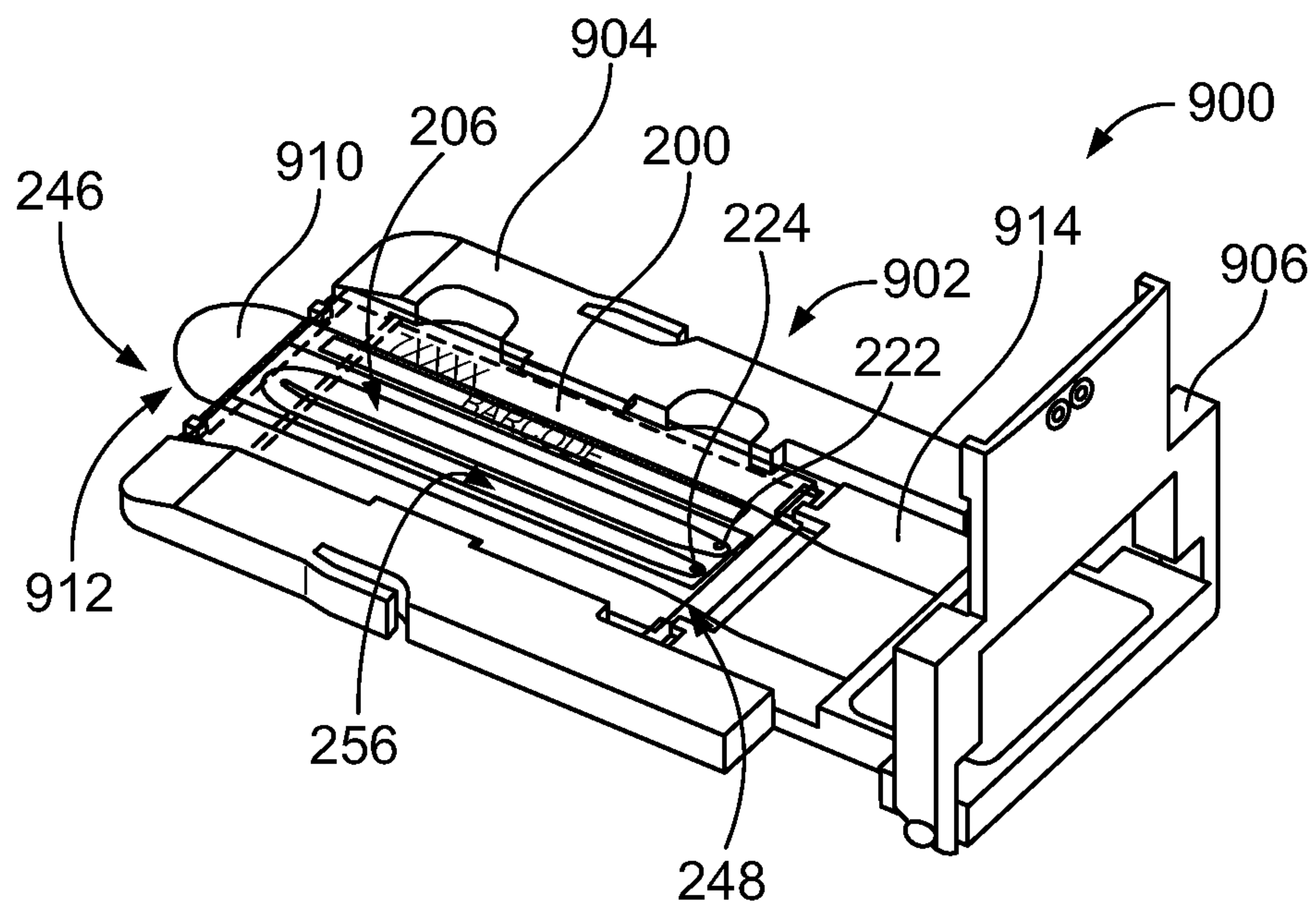


FIG. 13



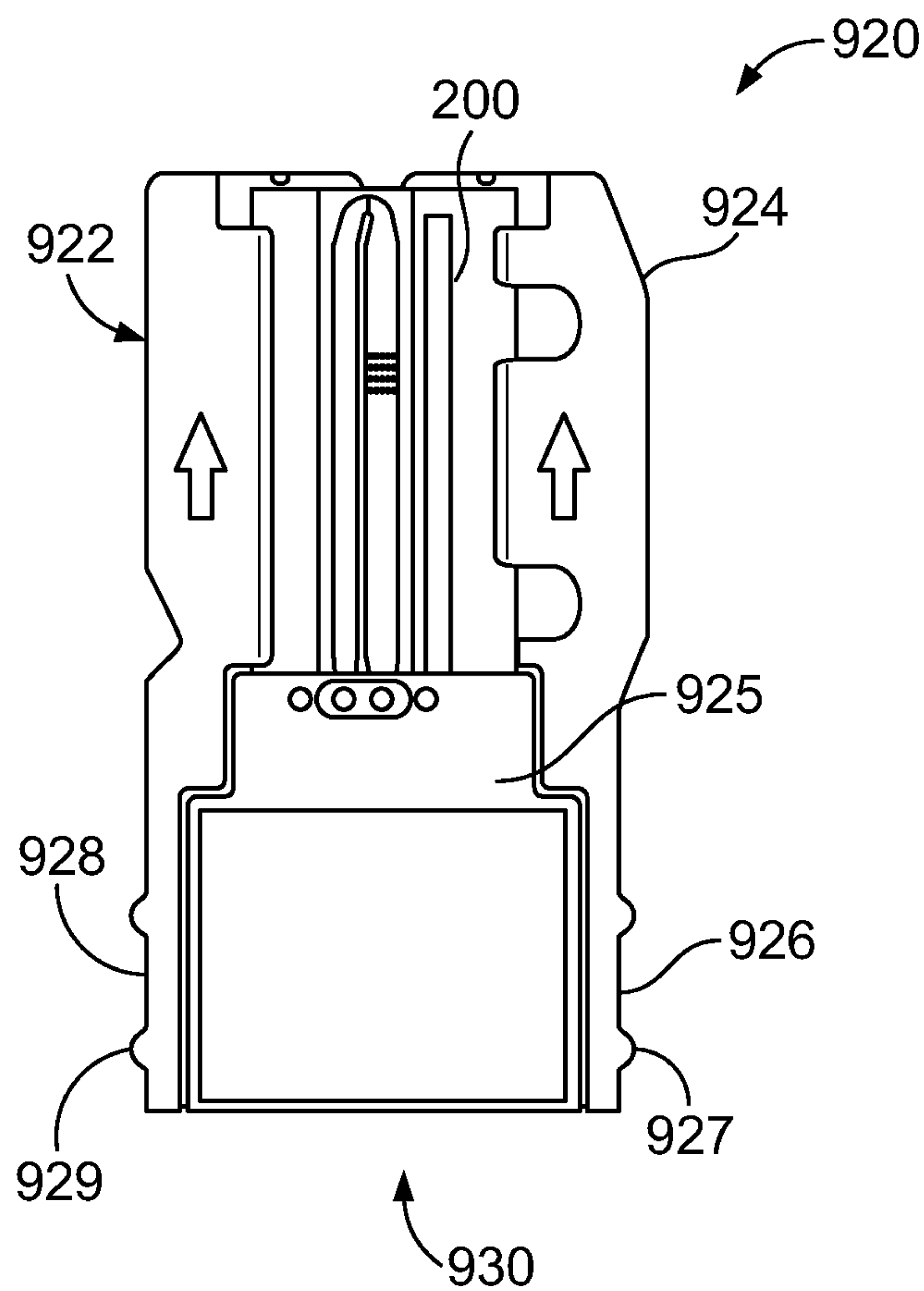


FIG. 14

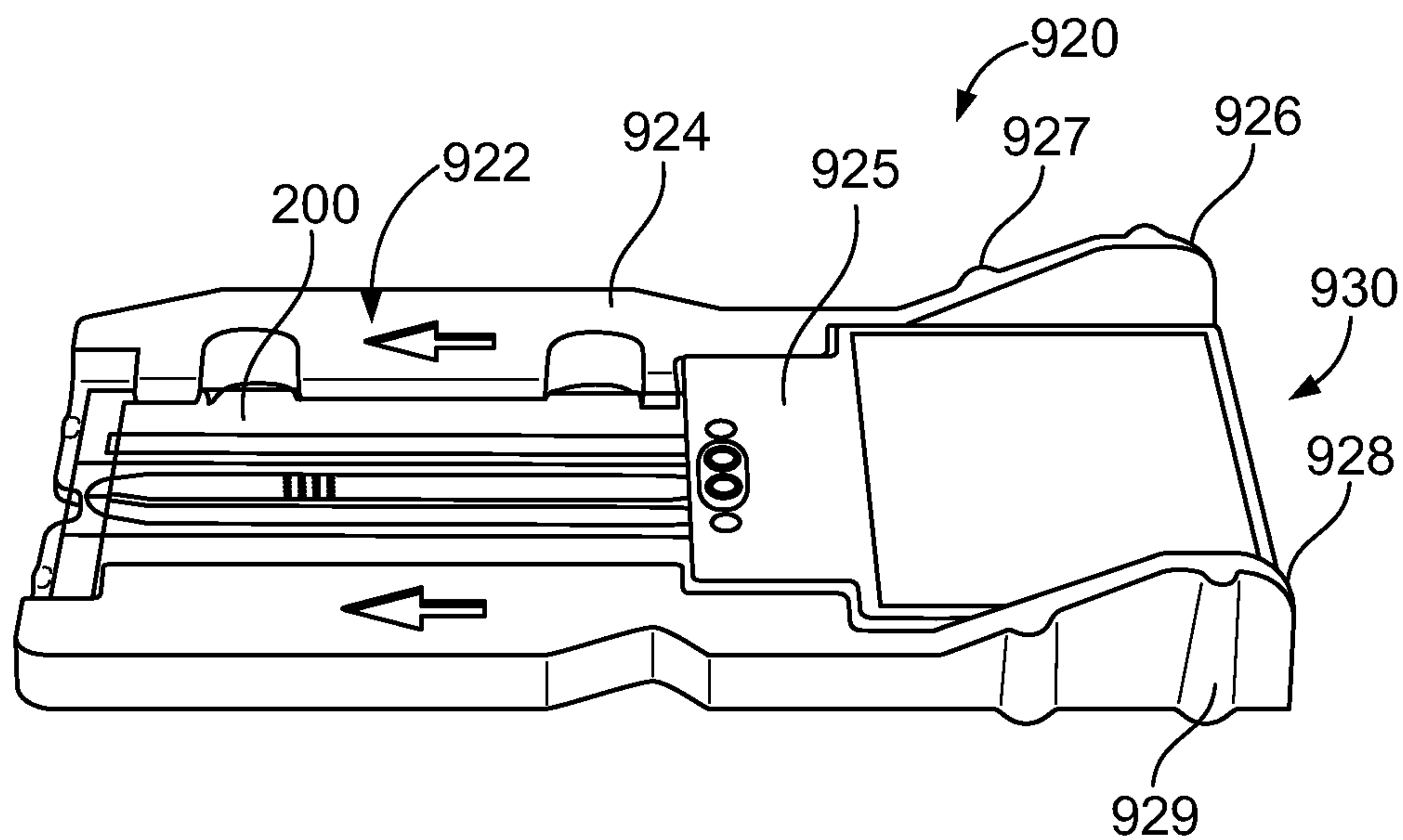


FIG. 15



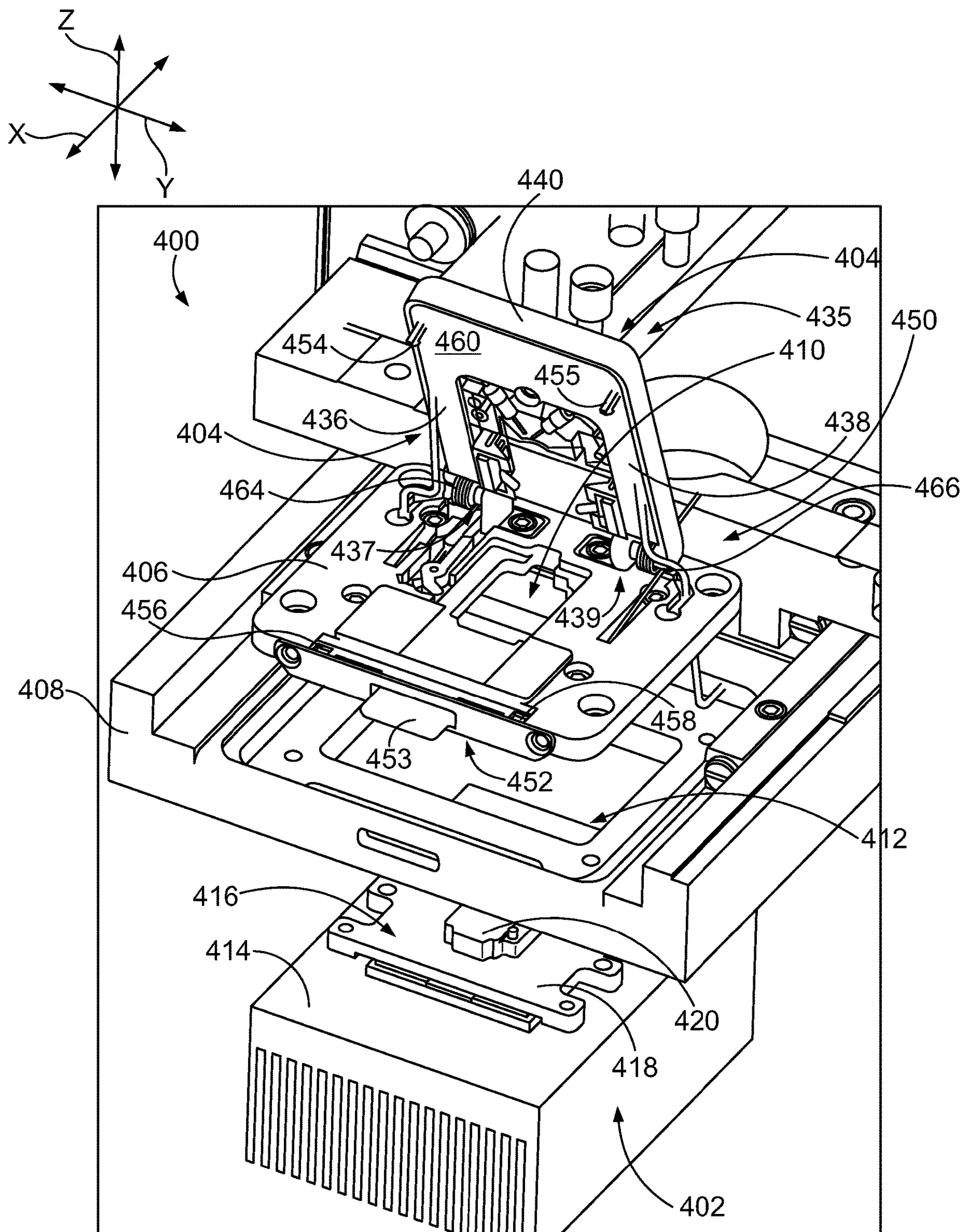
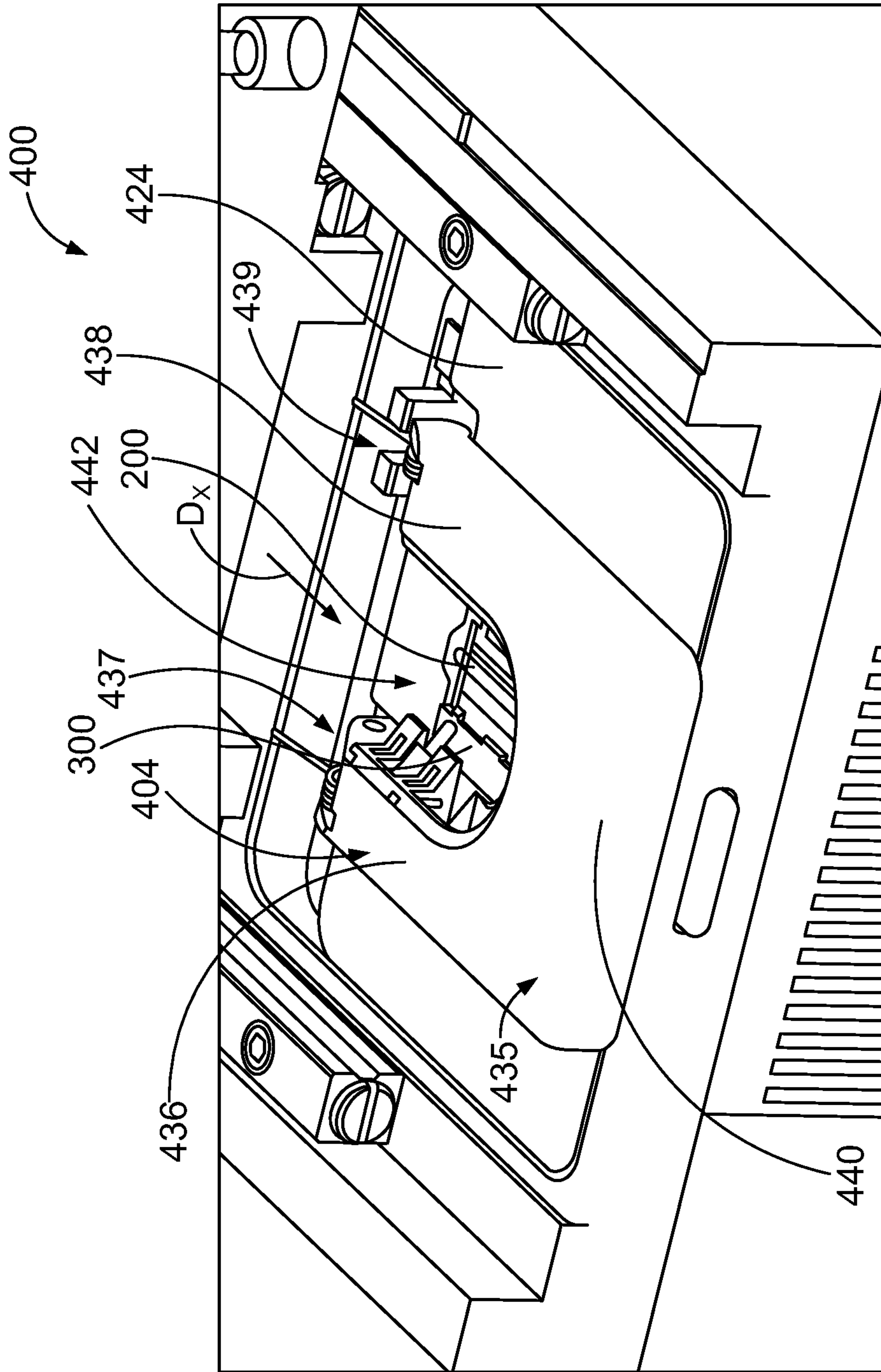


FIG. 16





**FIG. 17**



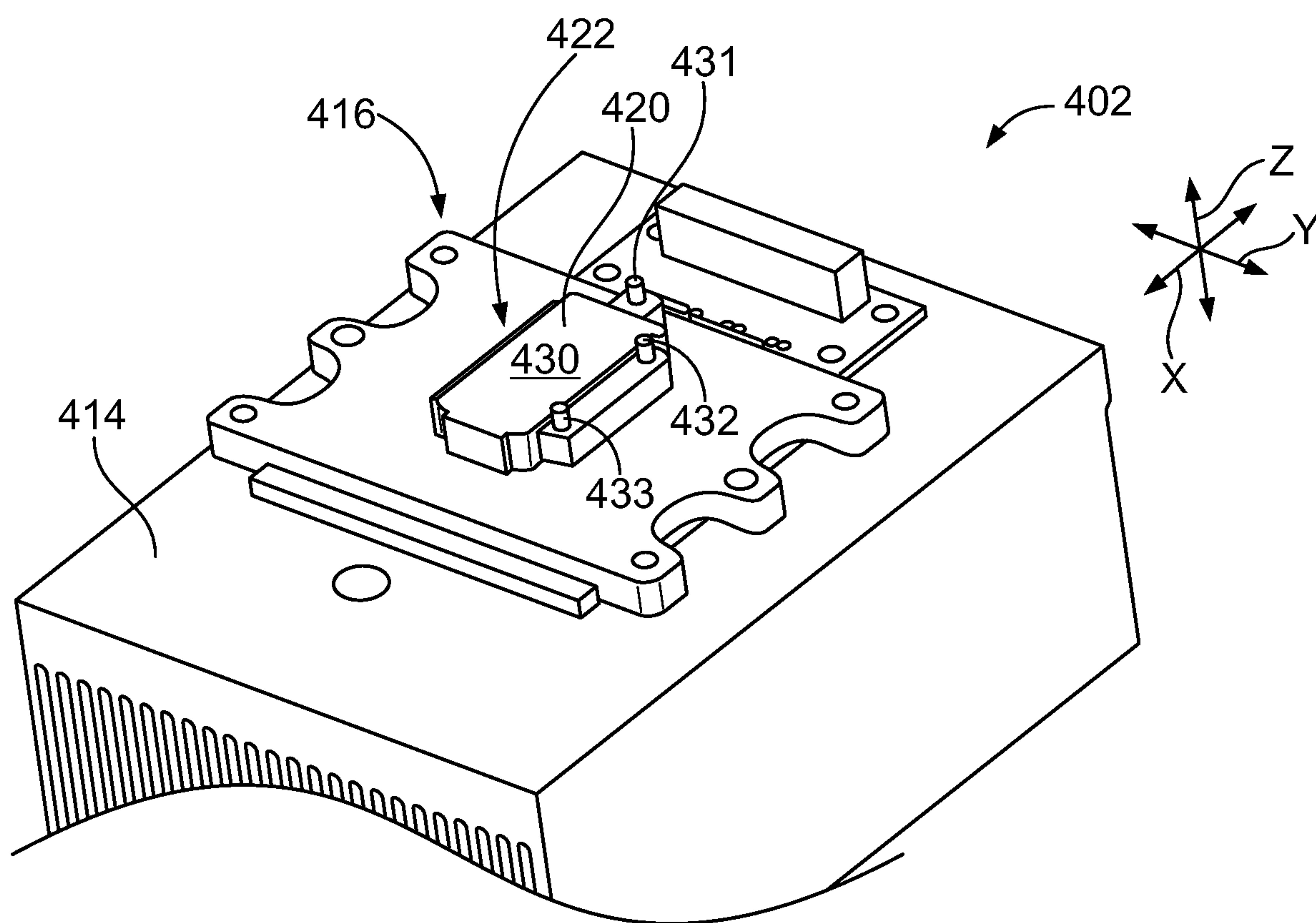


FIG. 18



FIG. 19



FIG. 20



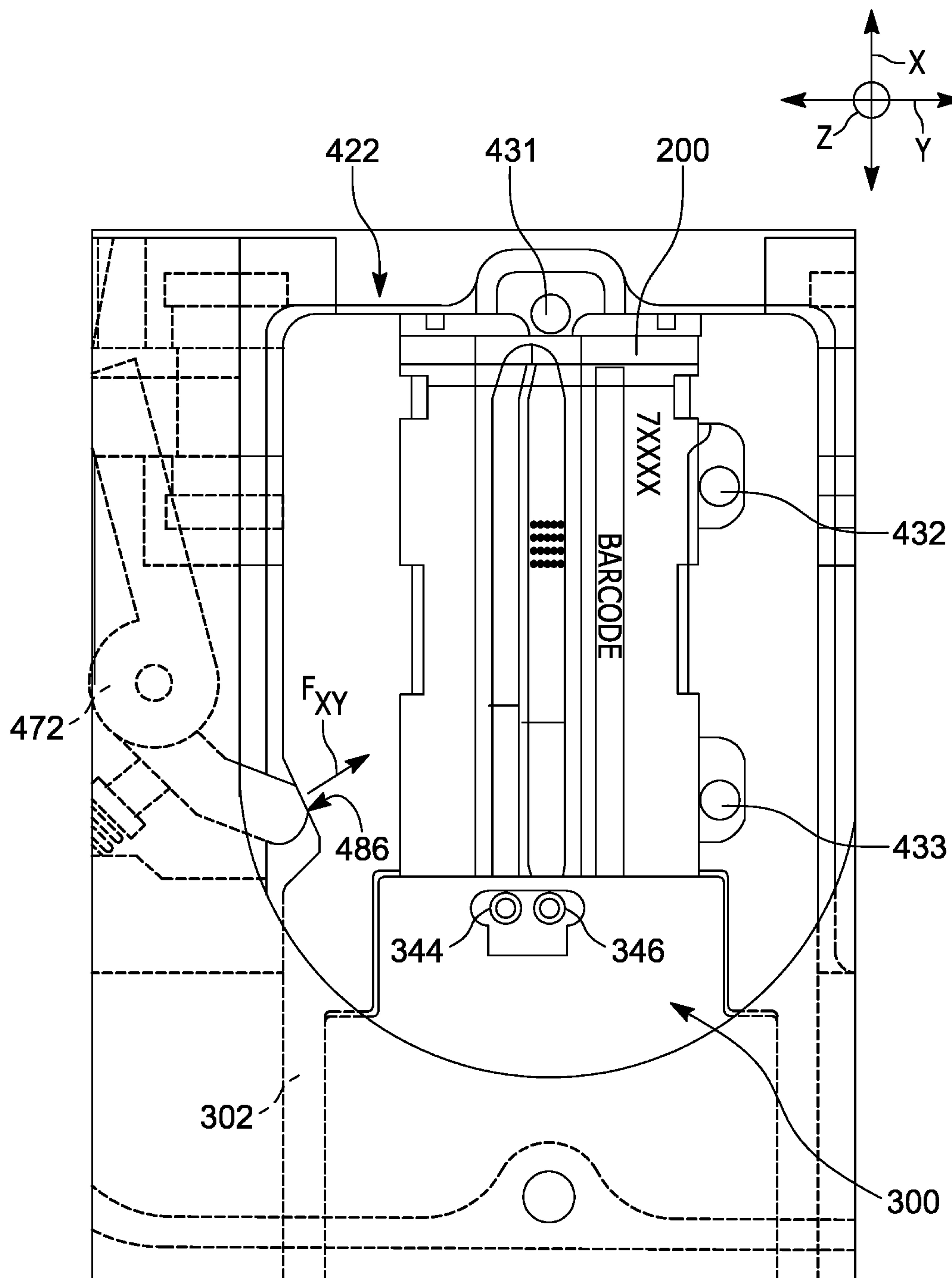


FIG. 21



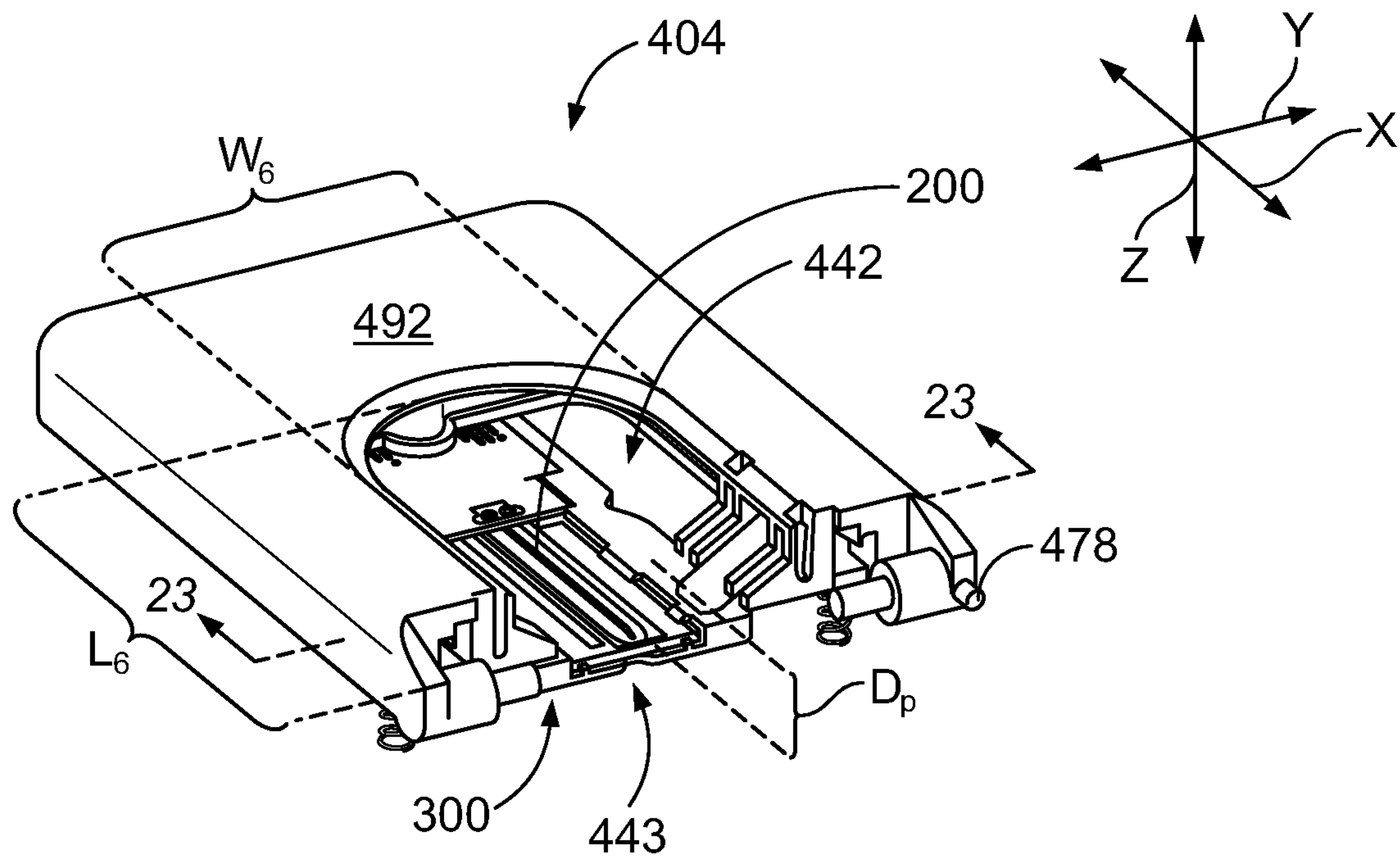


FIG. 22

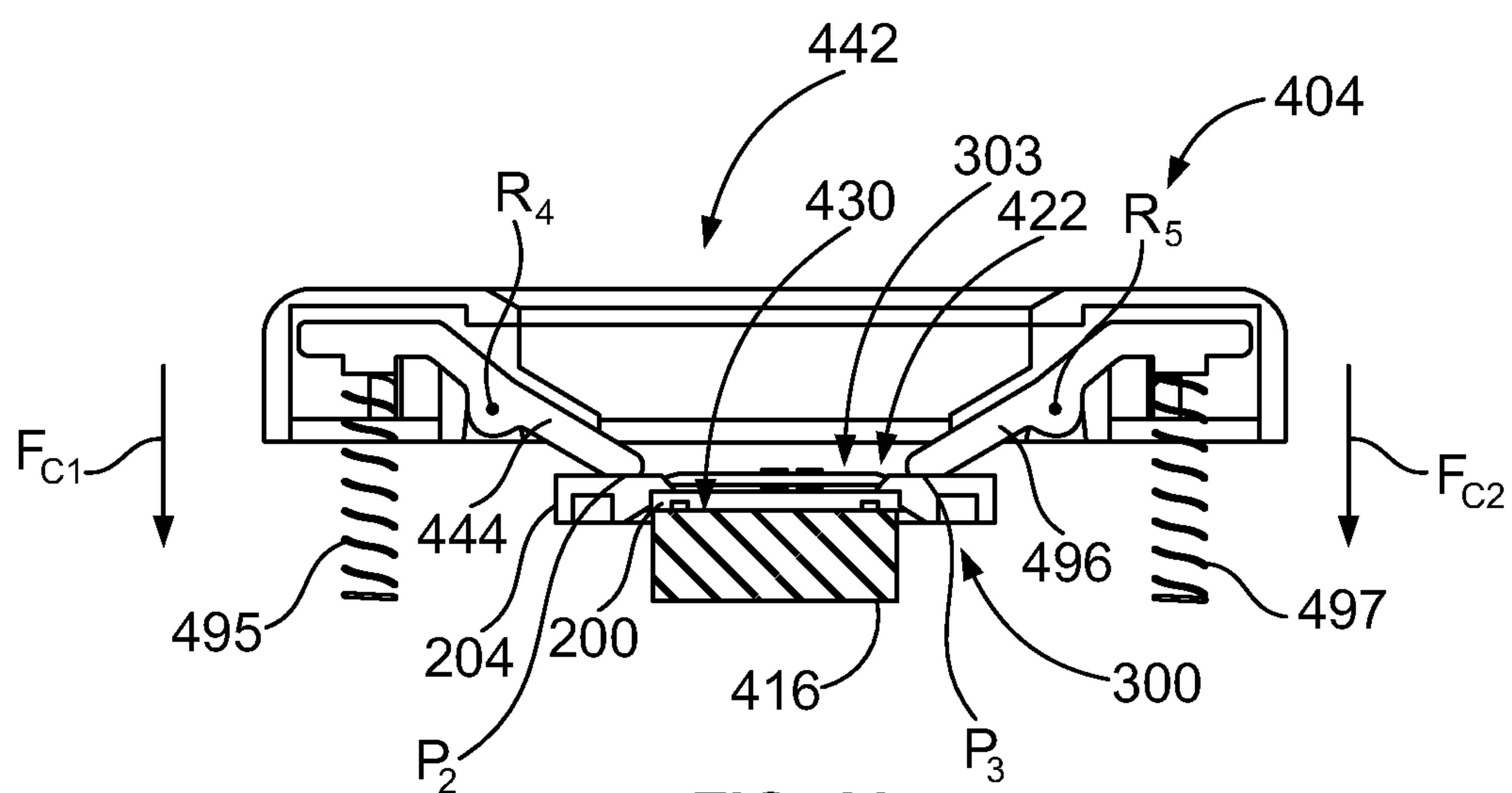


FIG. 23



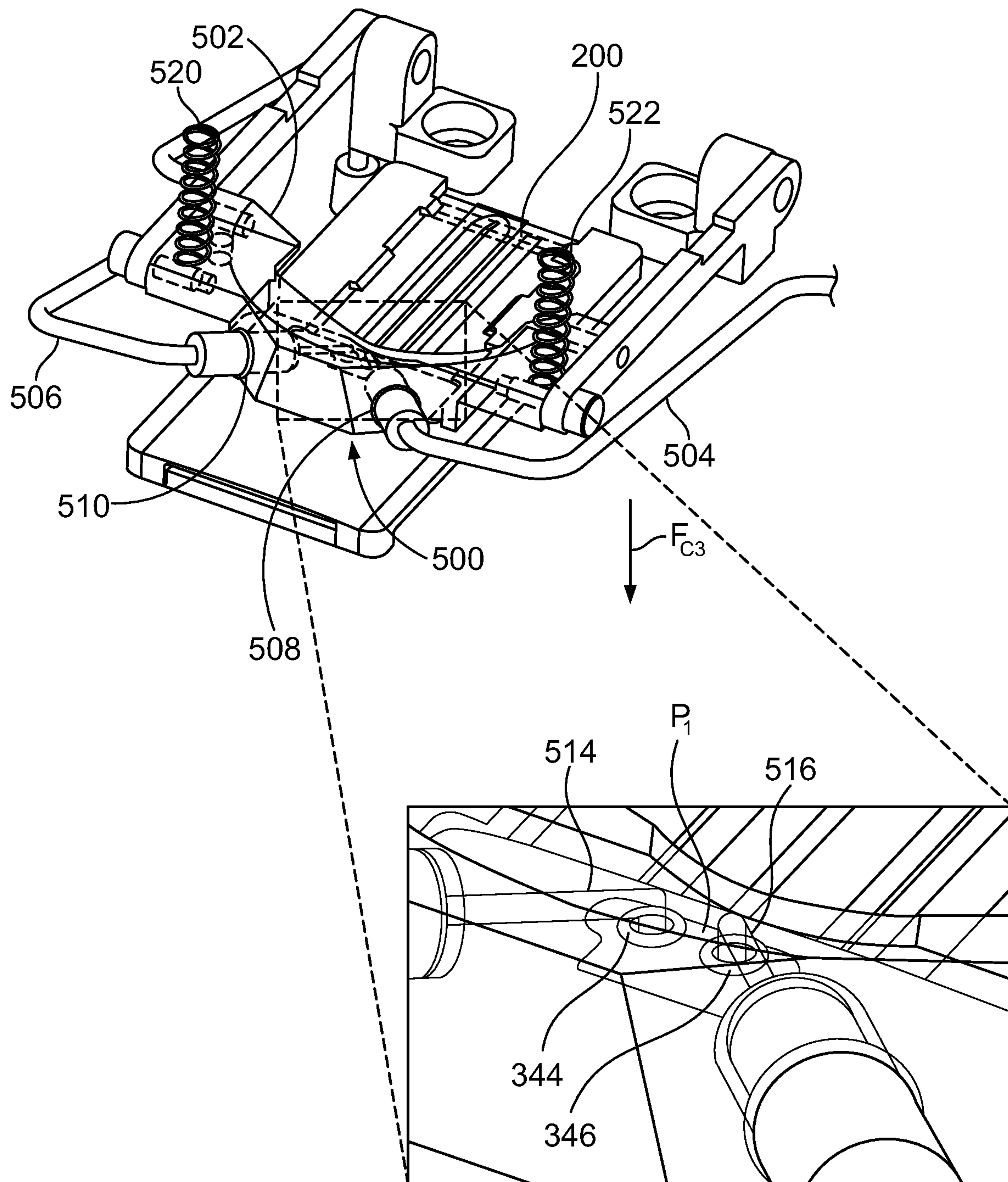


FIG. 24



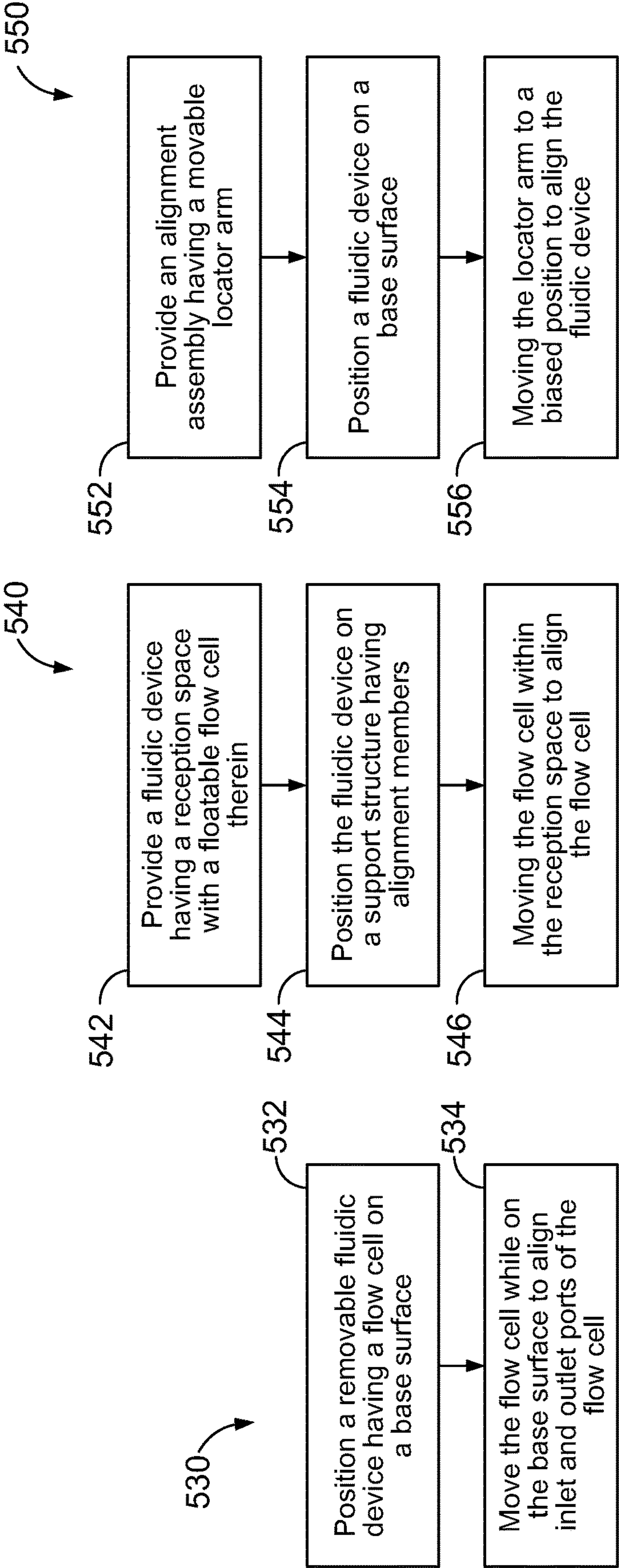


FIG. 25

FIG. 26

FIG. 27



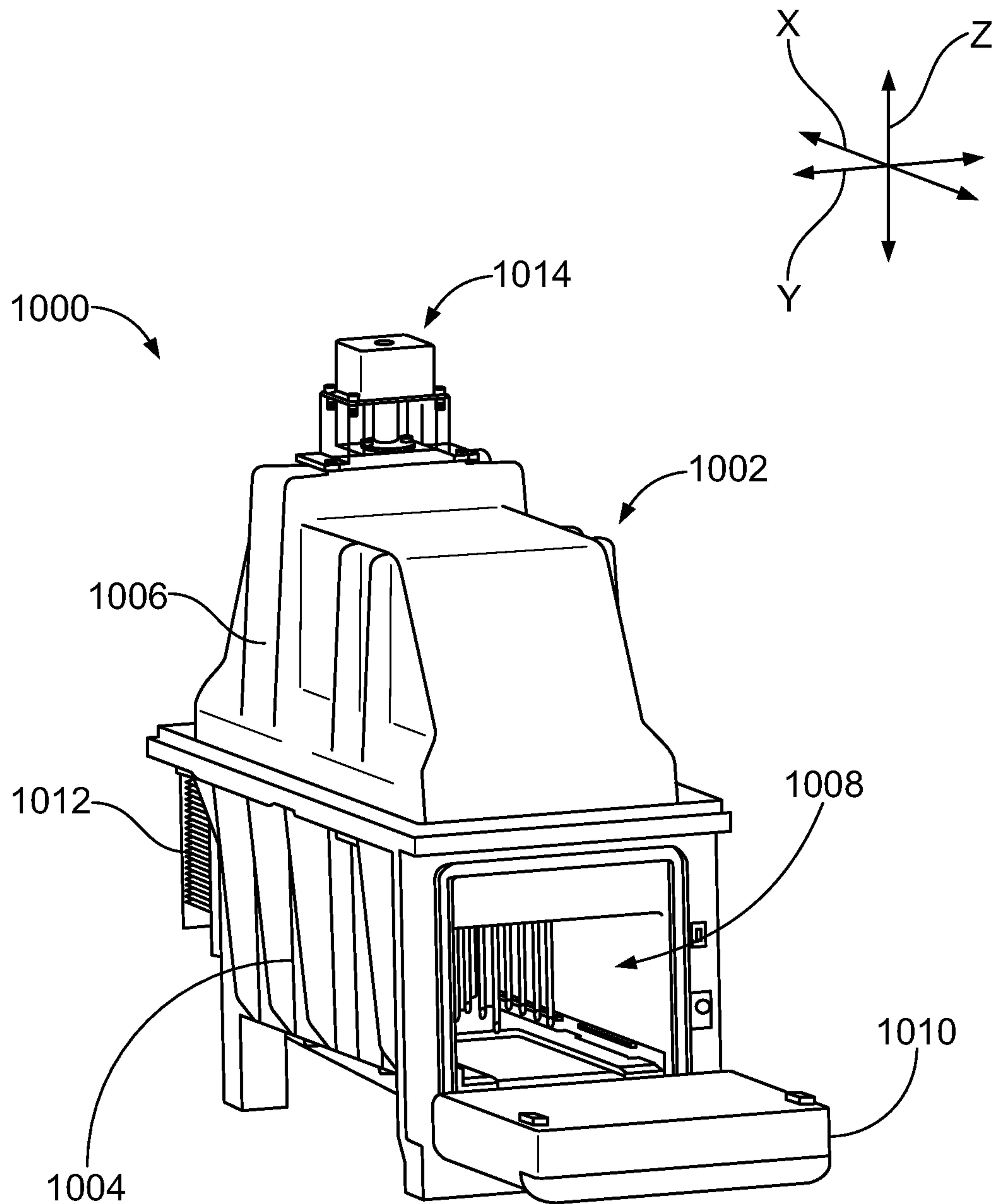


FIG. 28



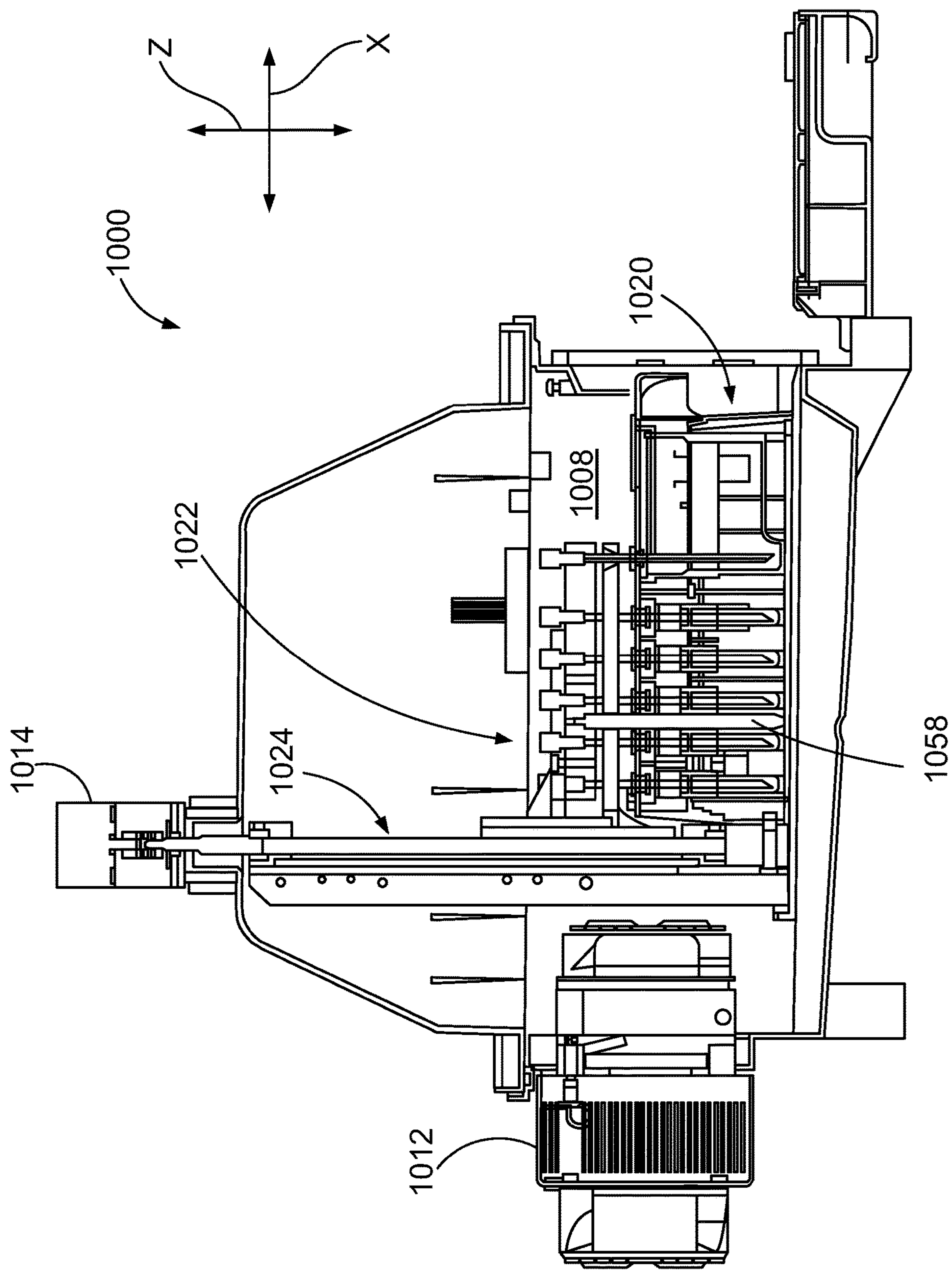


FIG. 29



**FIG. 30**



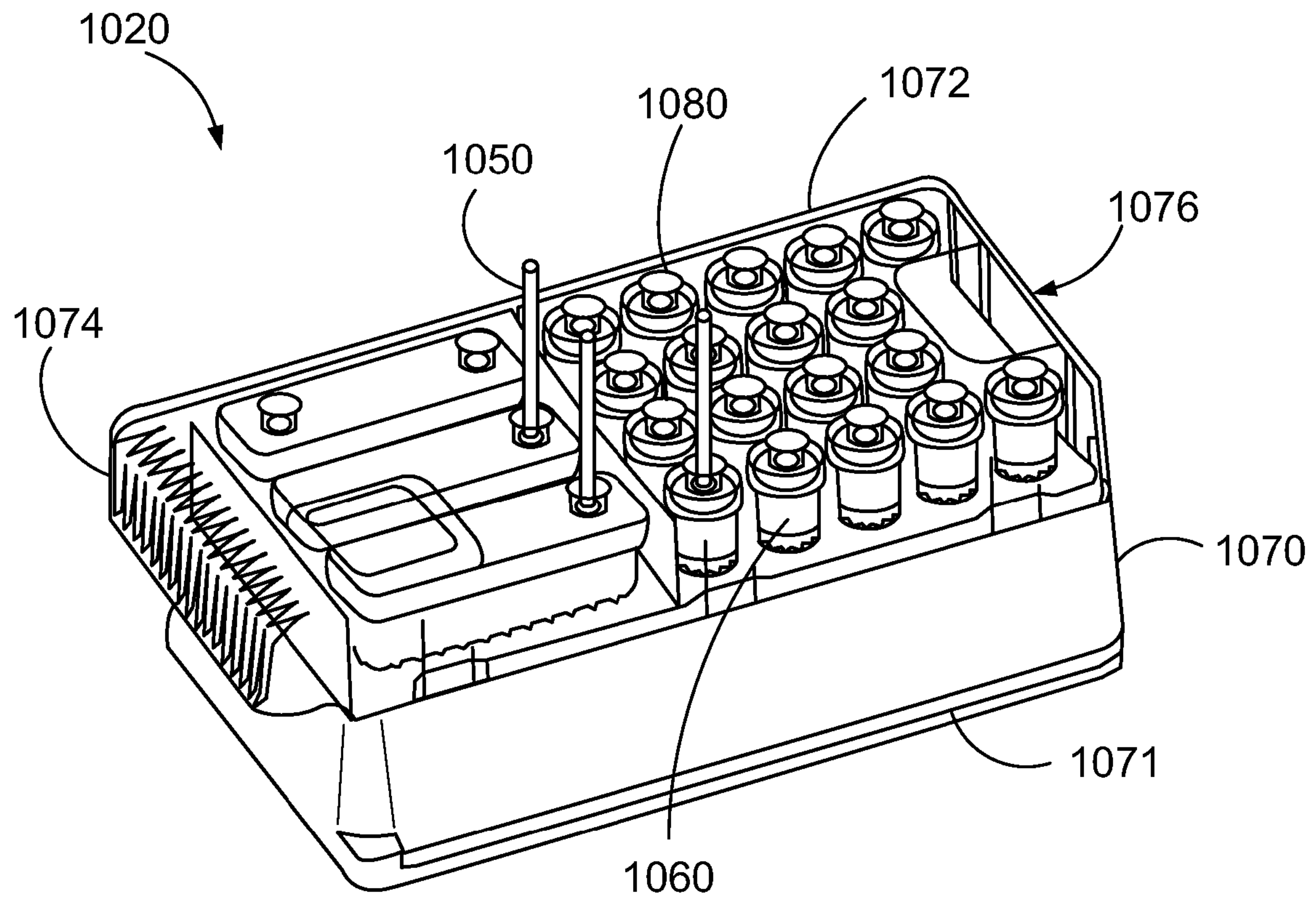


FIG. 31

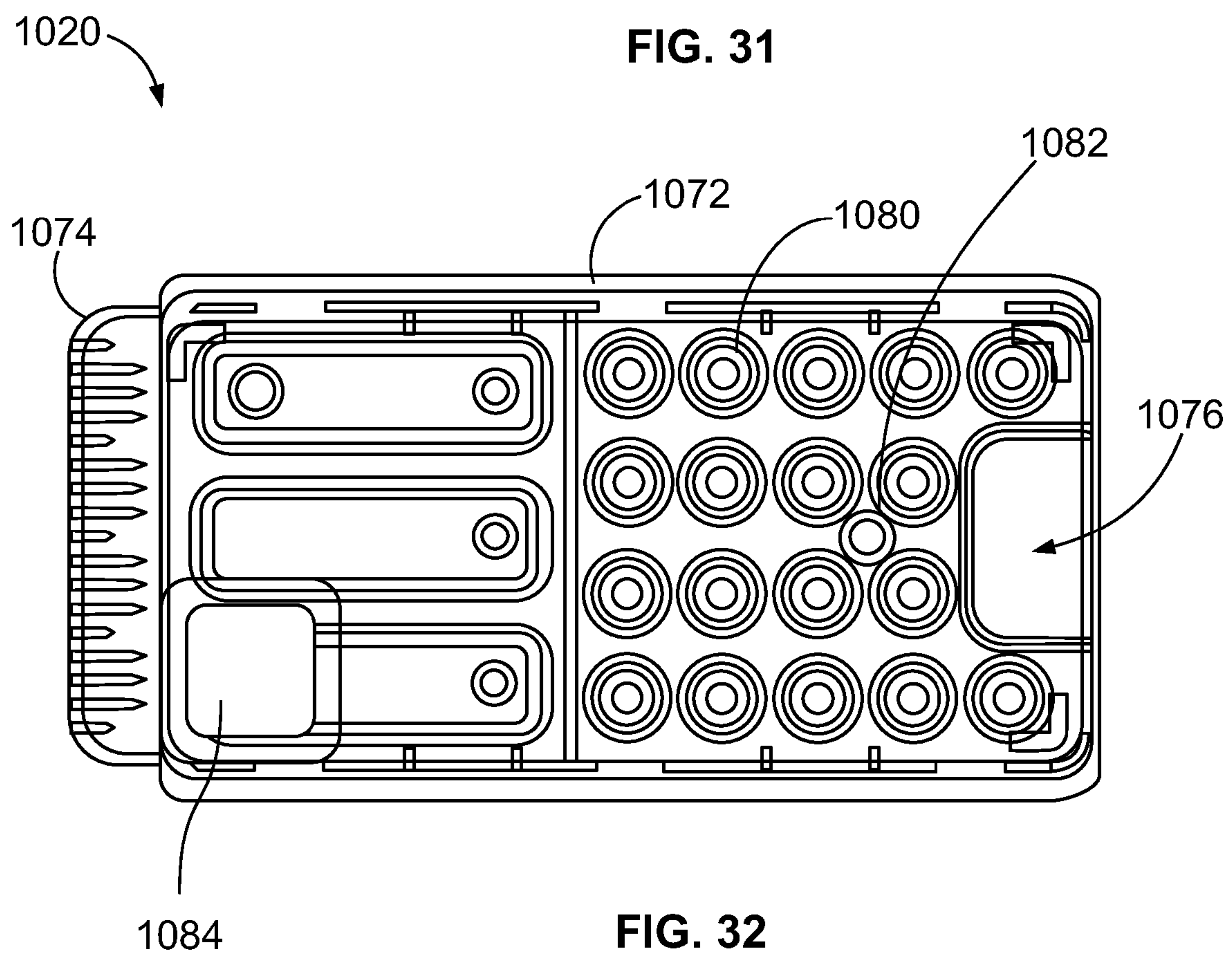


FIG. 32



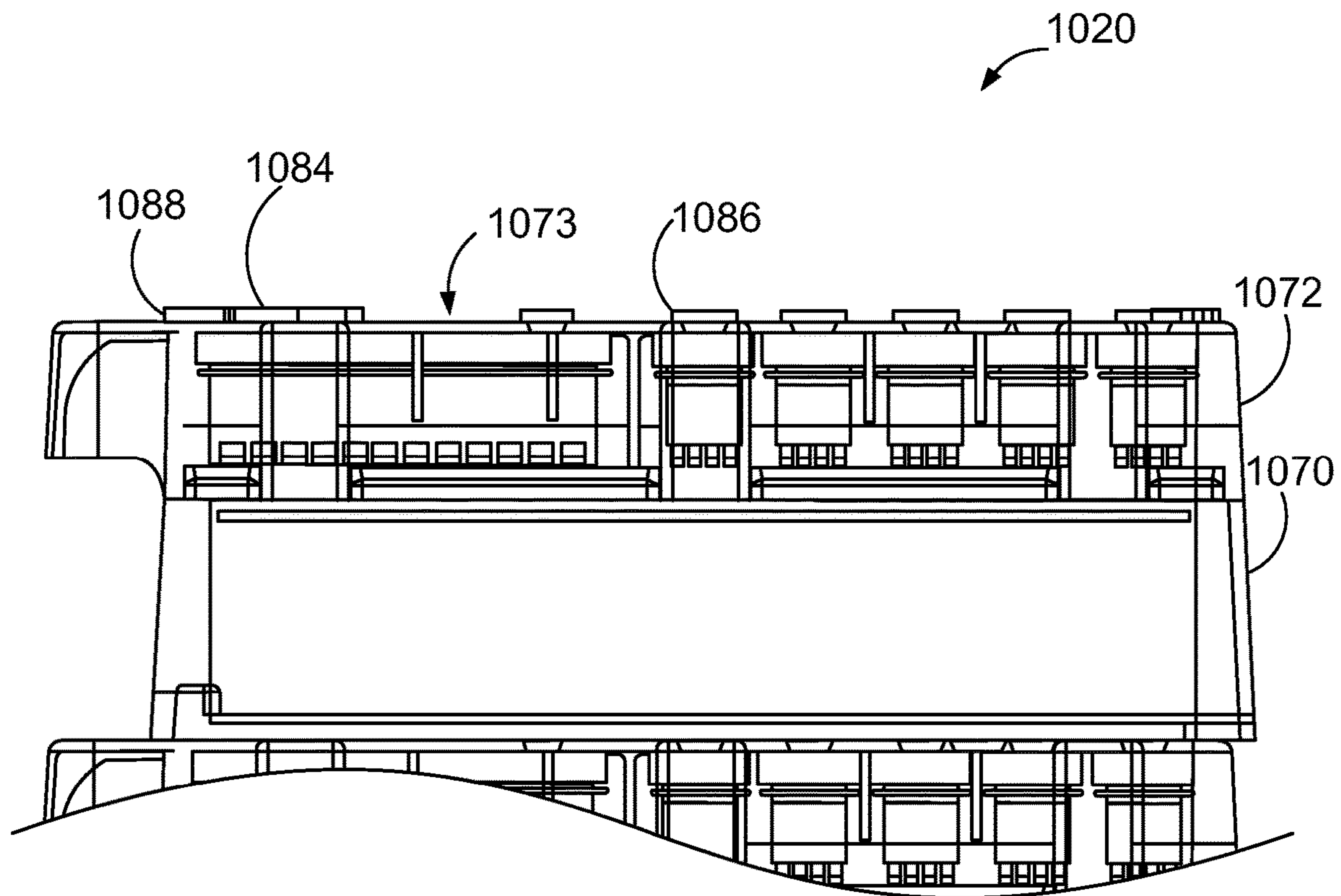


FIG. 33

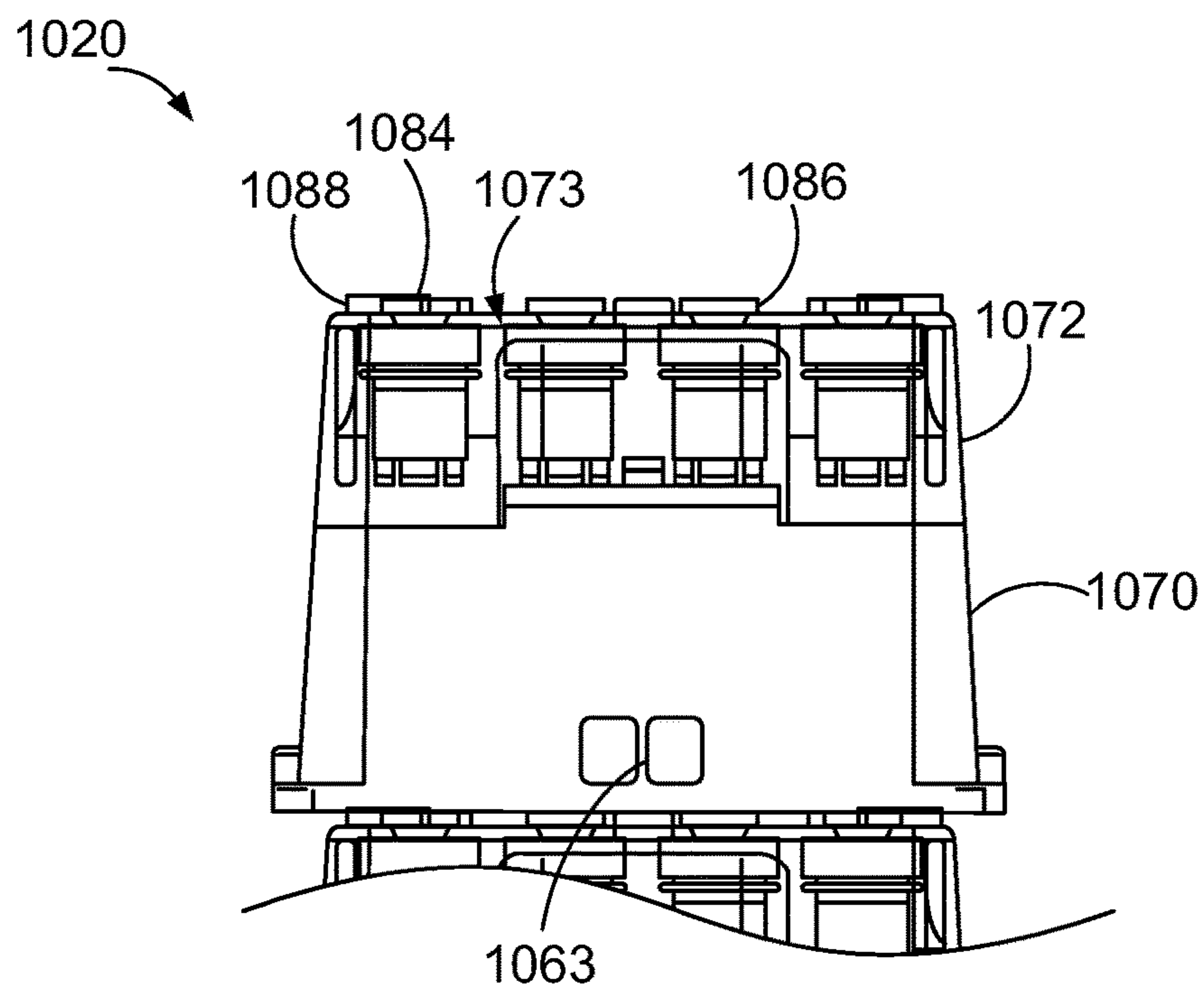


FIG. 34



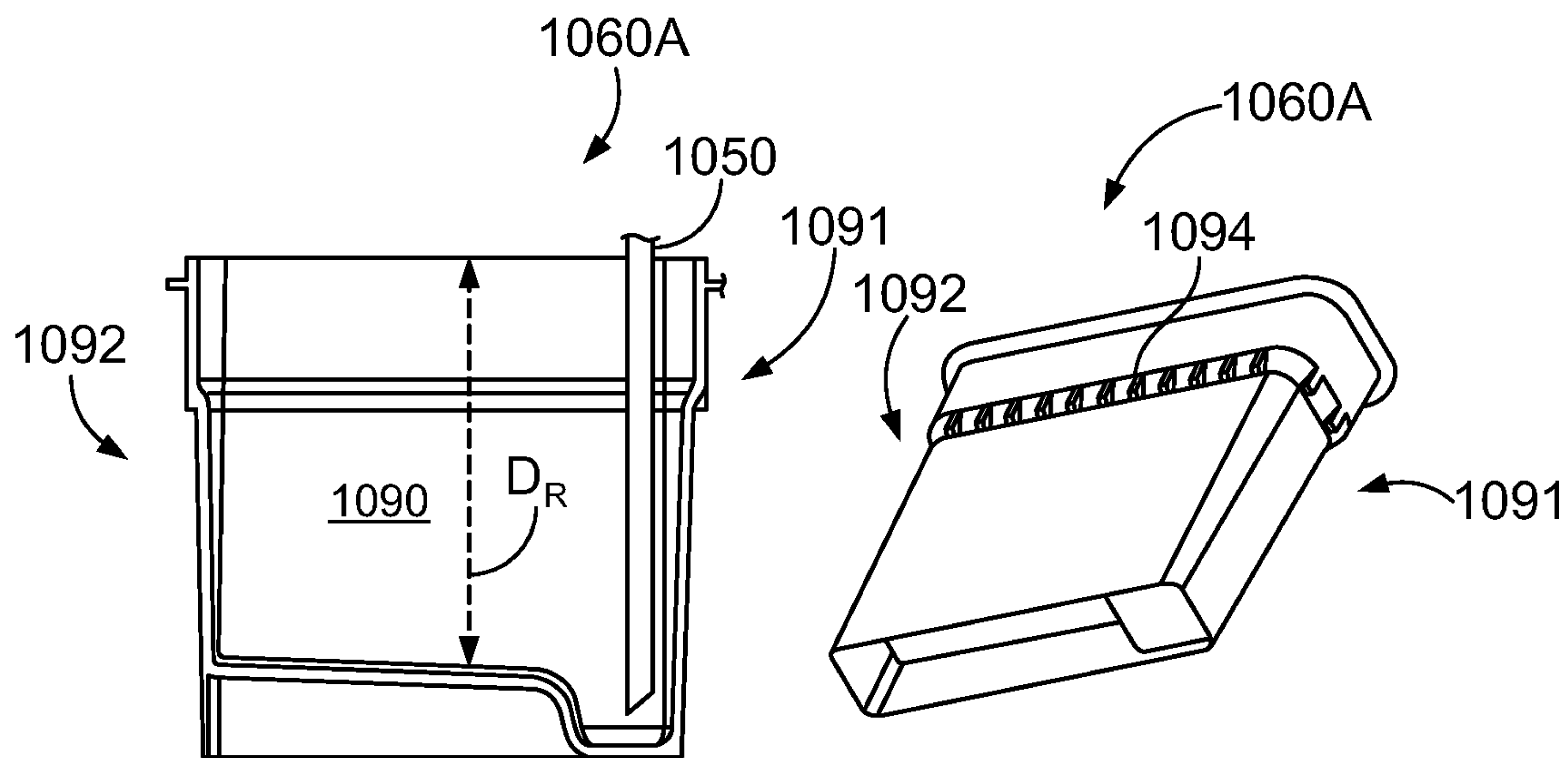


FIG. 36

FIG. 35

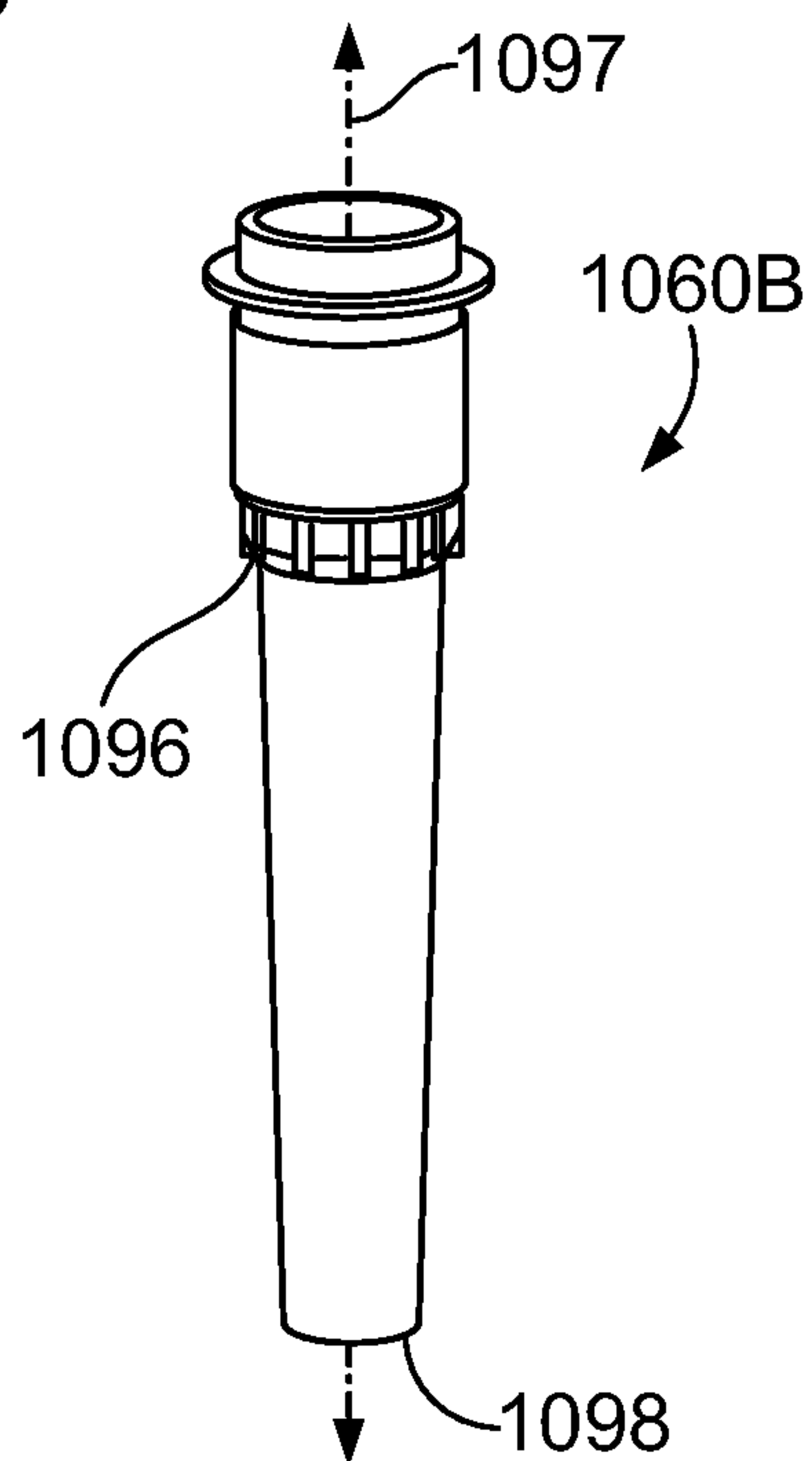


FIG. 37



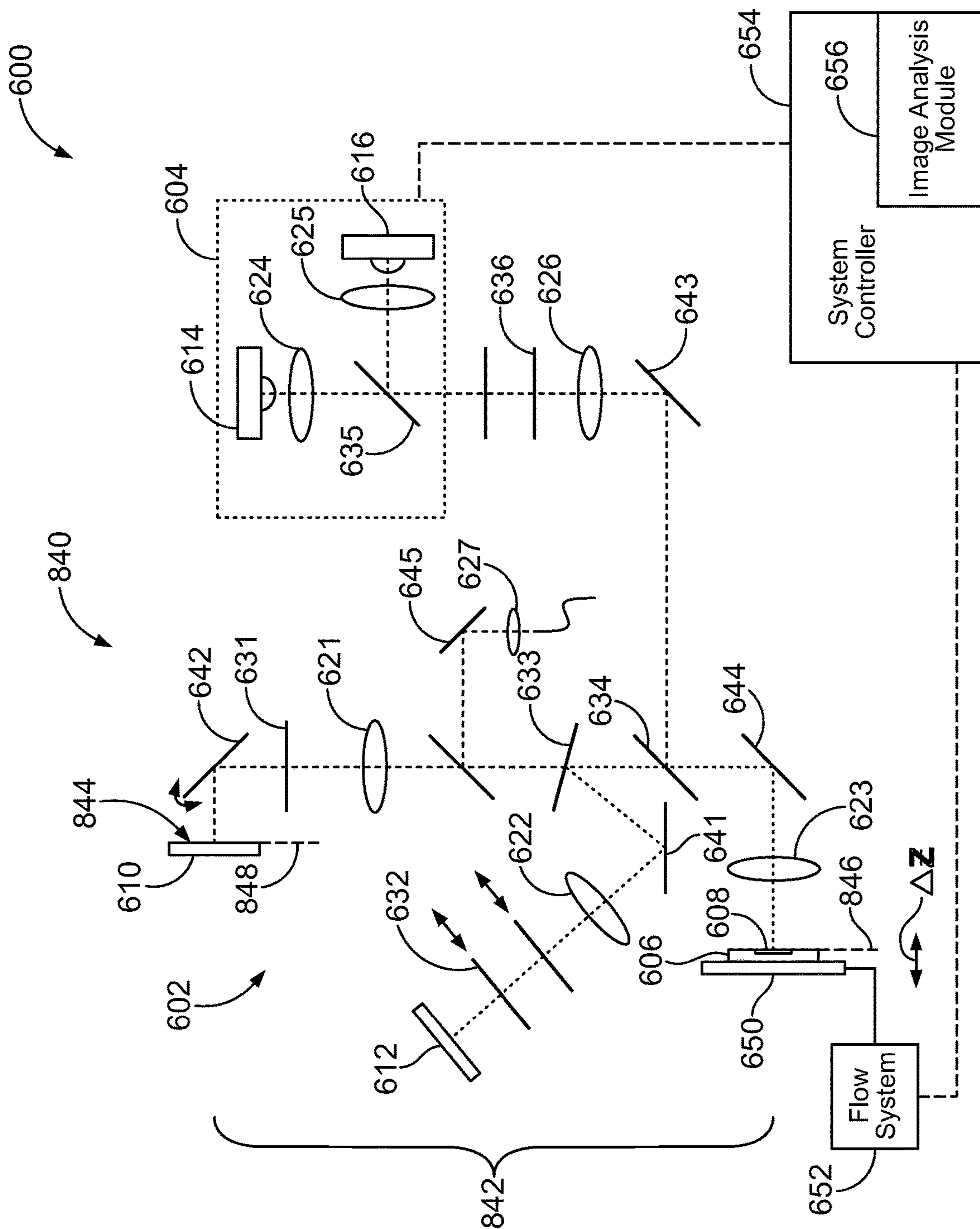


FIG. 38



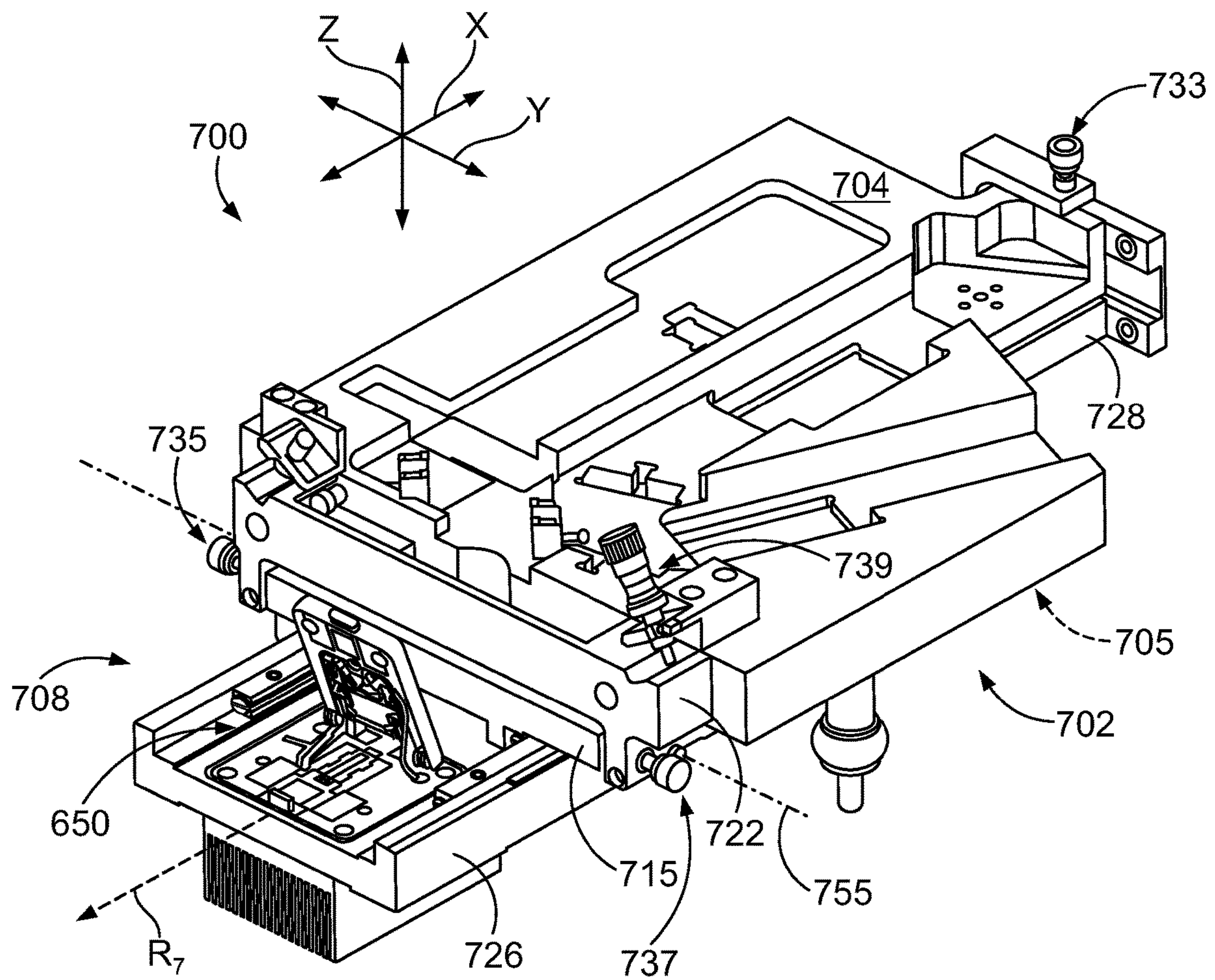


FIG. 39



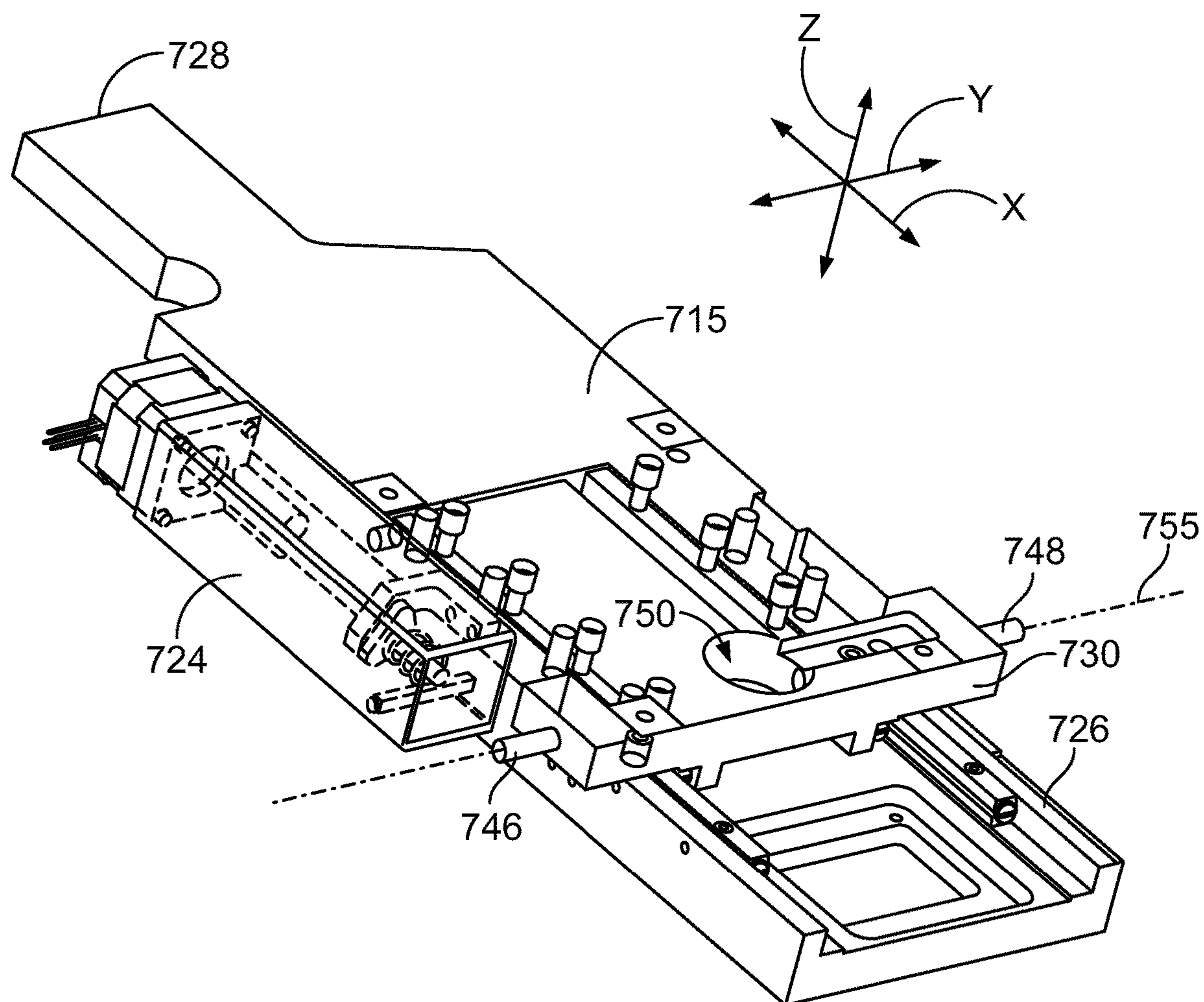


FIG. 40



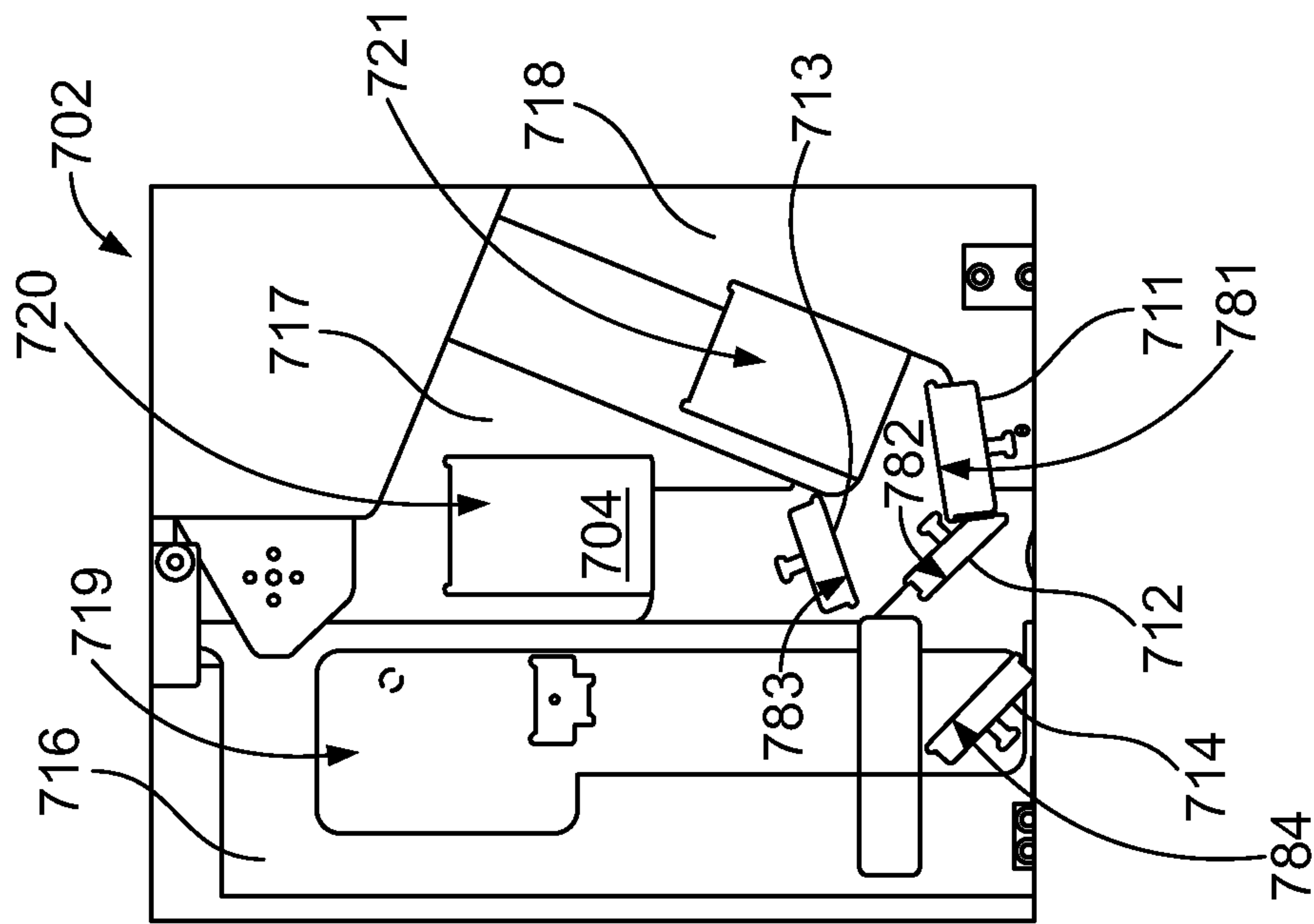


FIG. 42

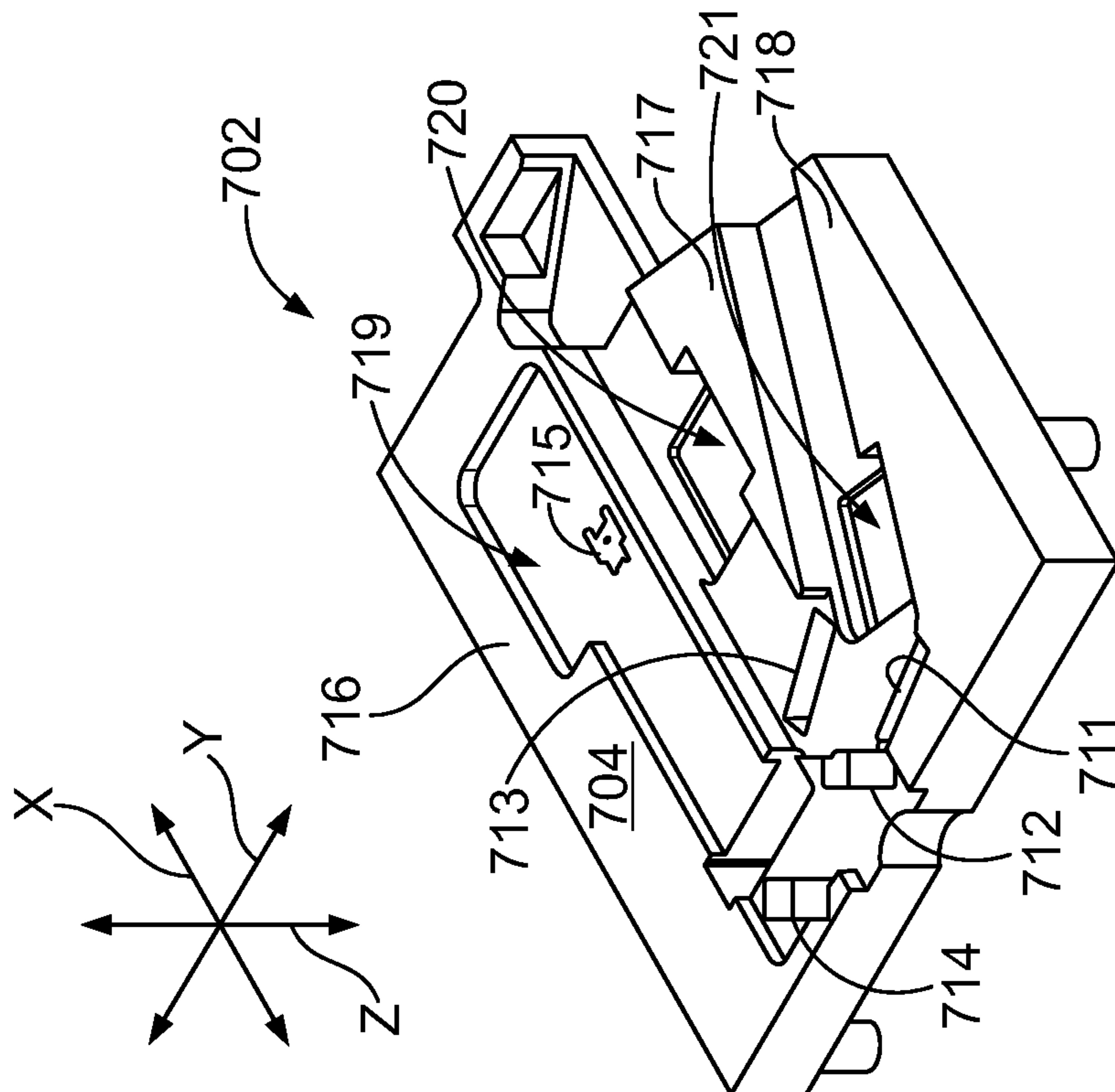
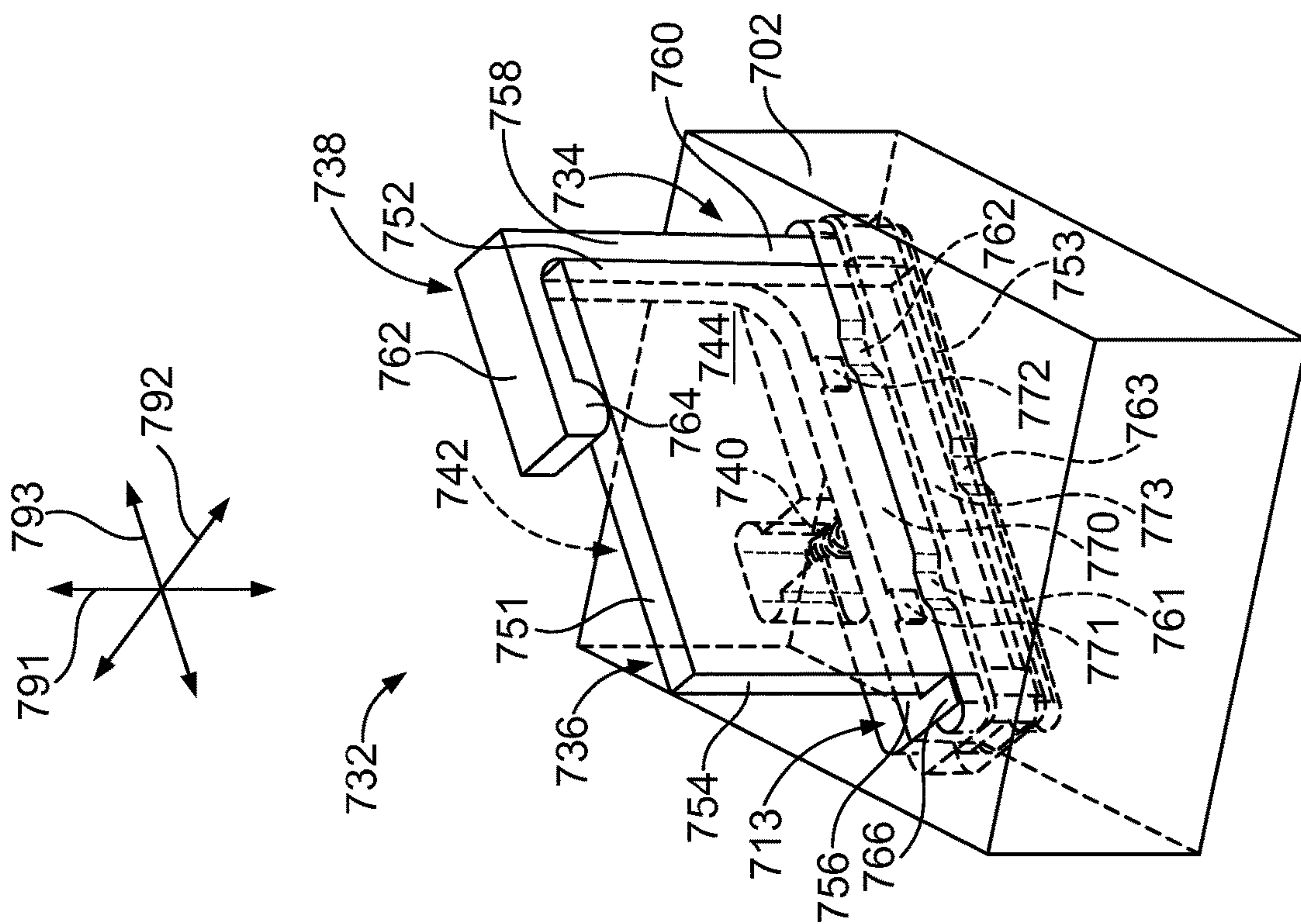
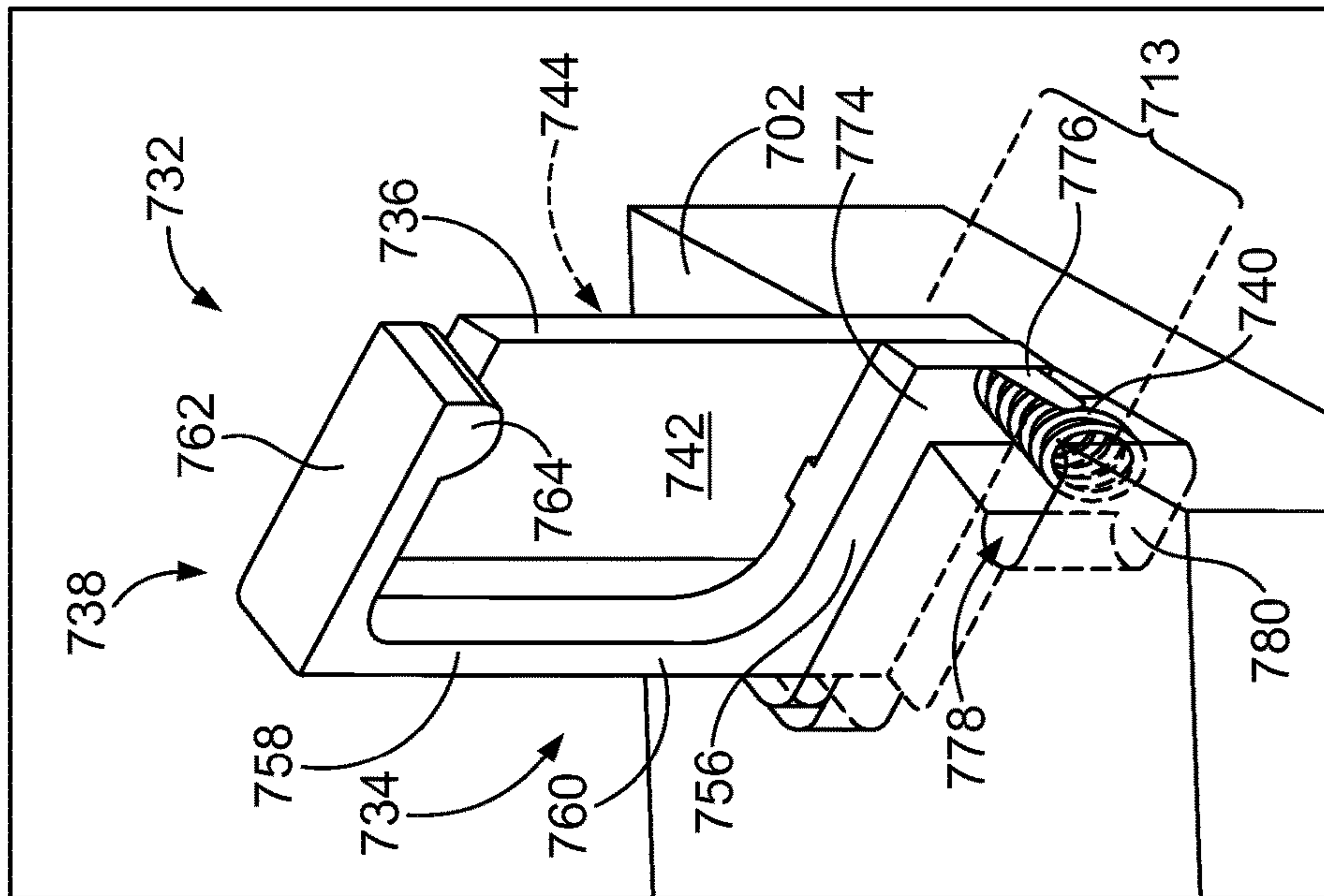


FIG. 41





**FIG. 43**



**FIG. 44**



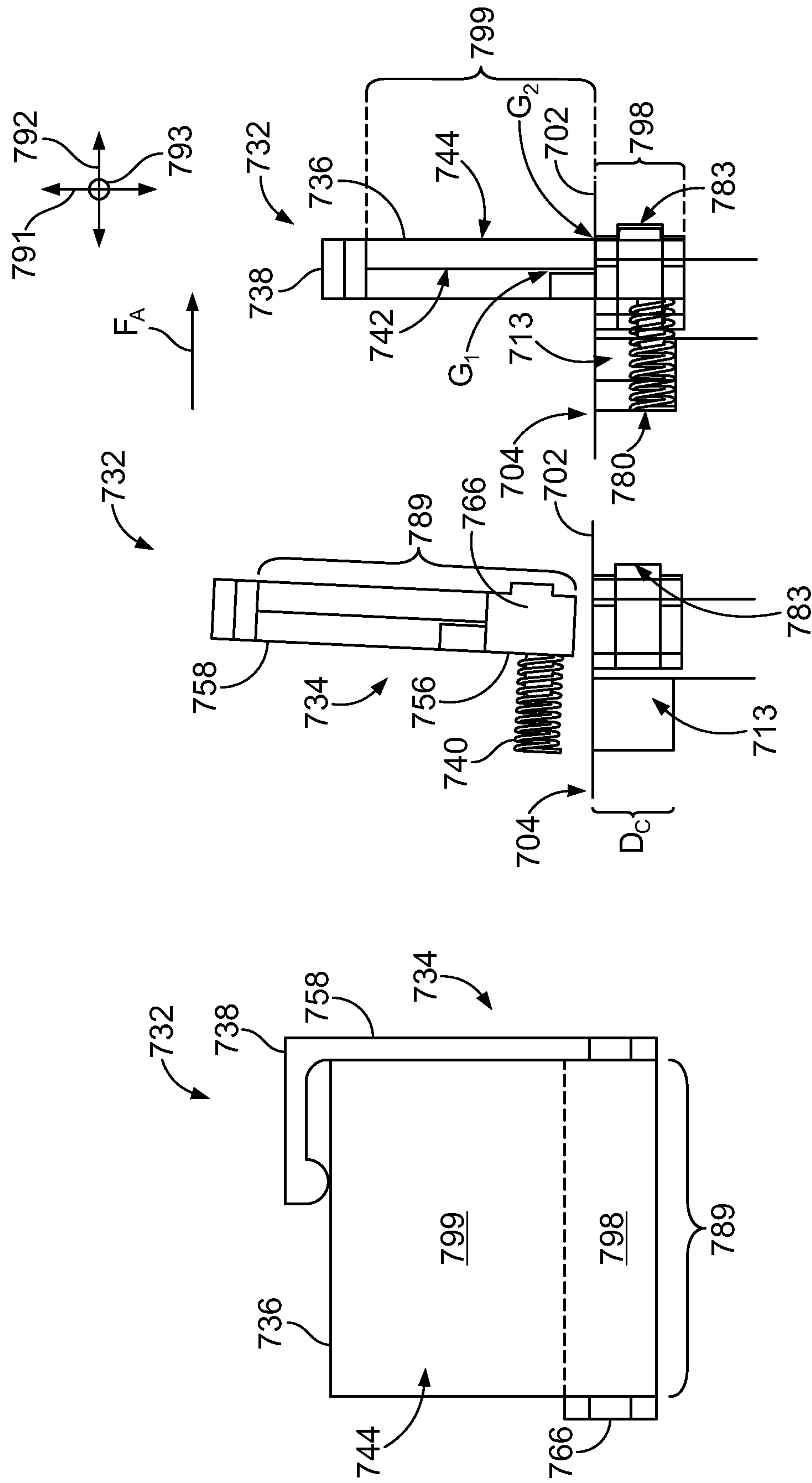
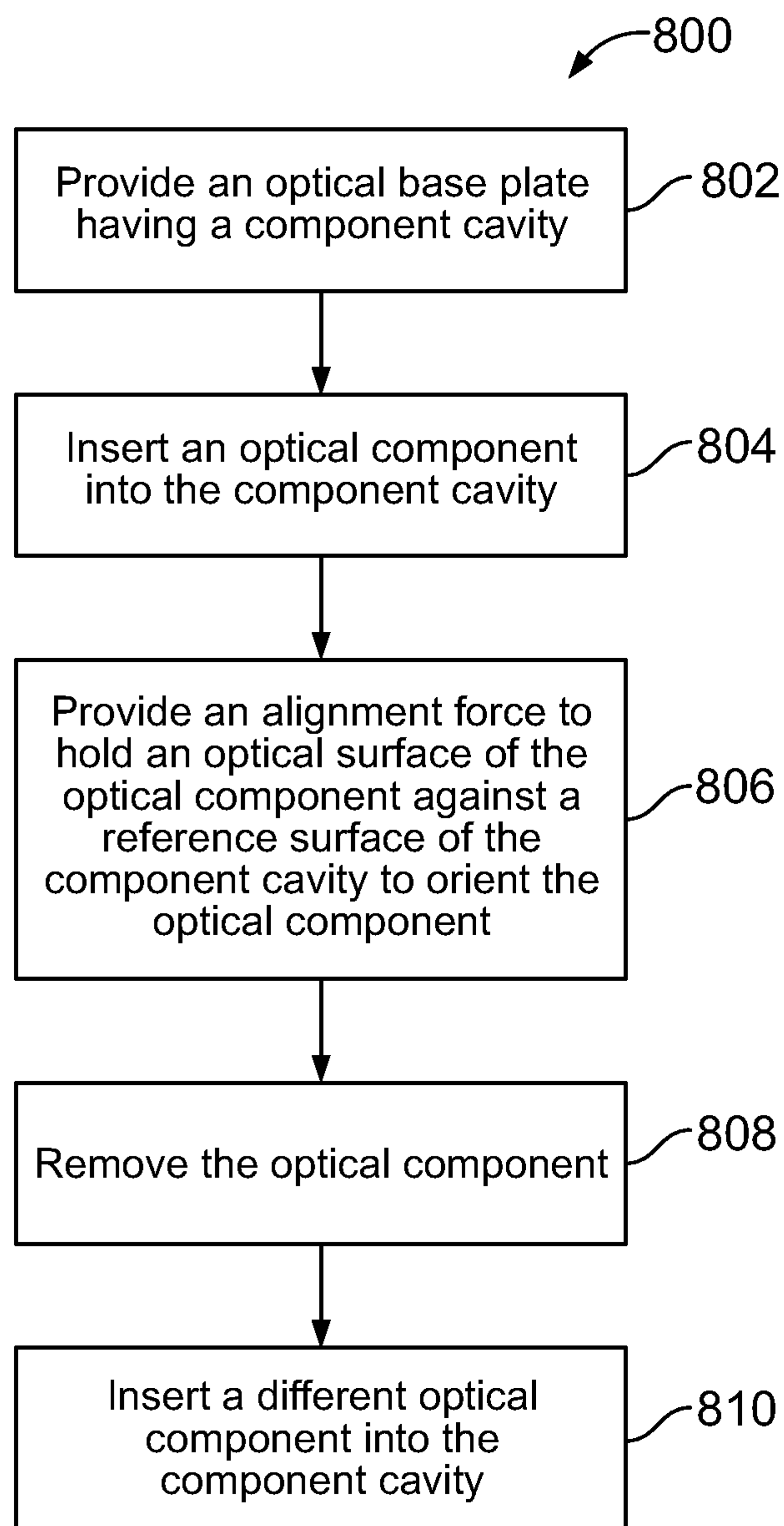


FIG. 46

FIG. 45



**FIG. 47**



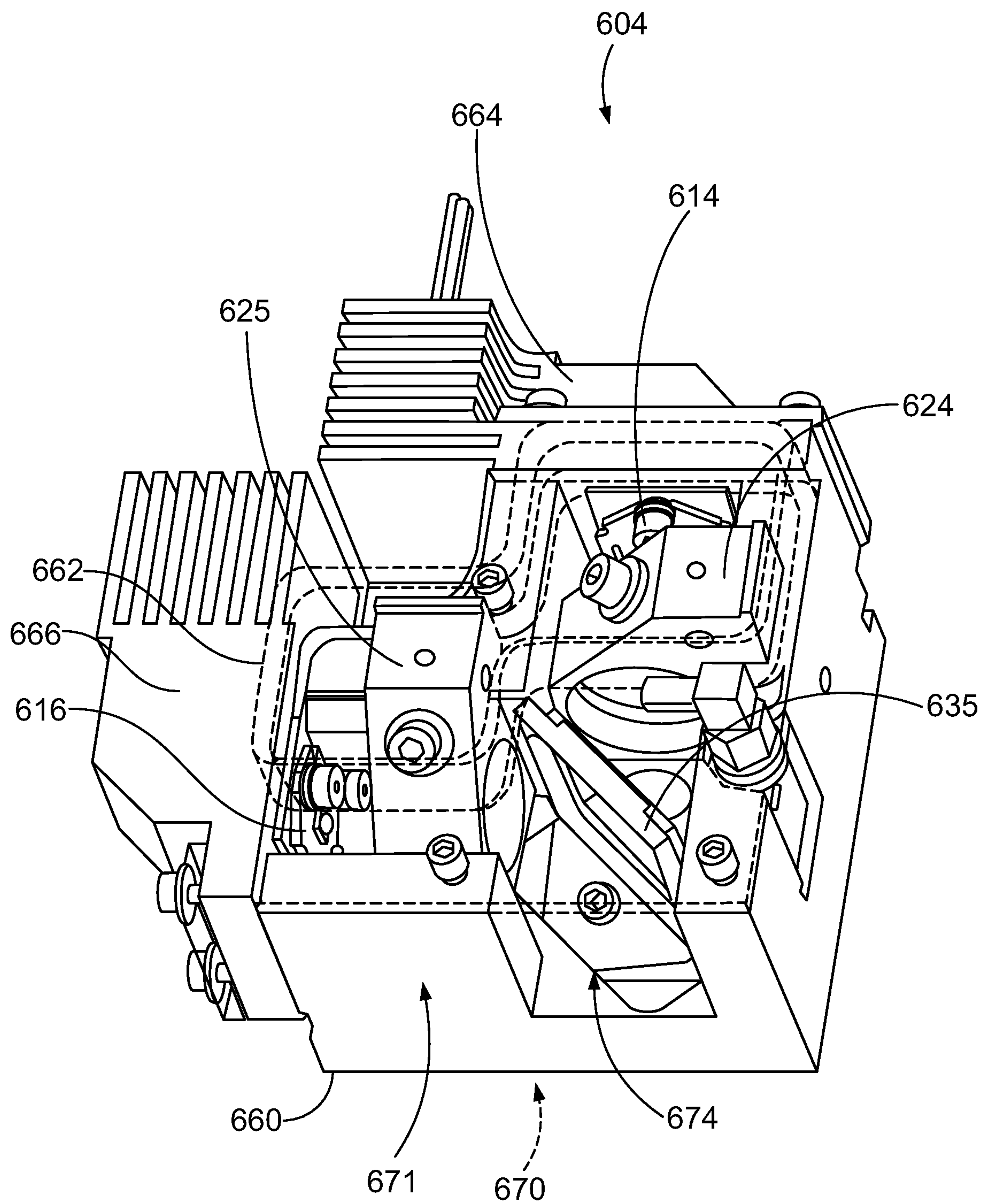


FIG.48



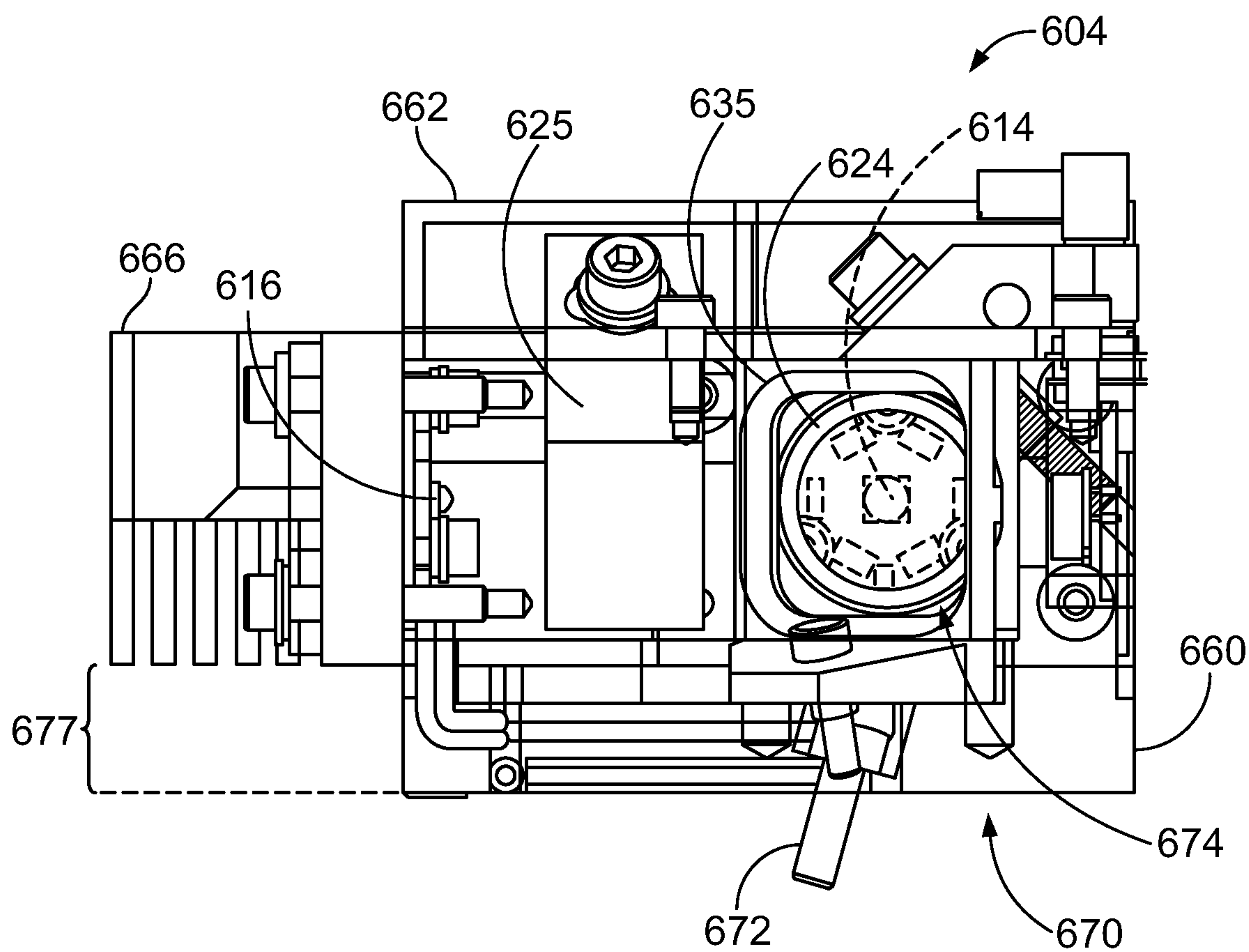


FIG. 49



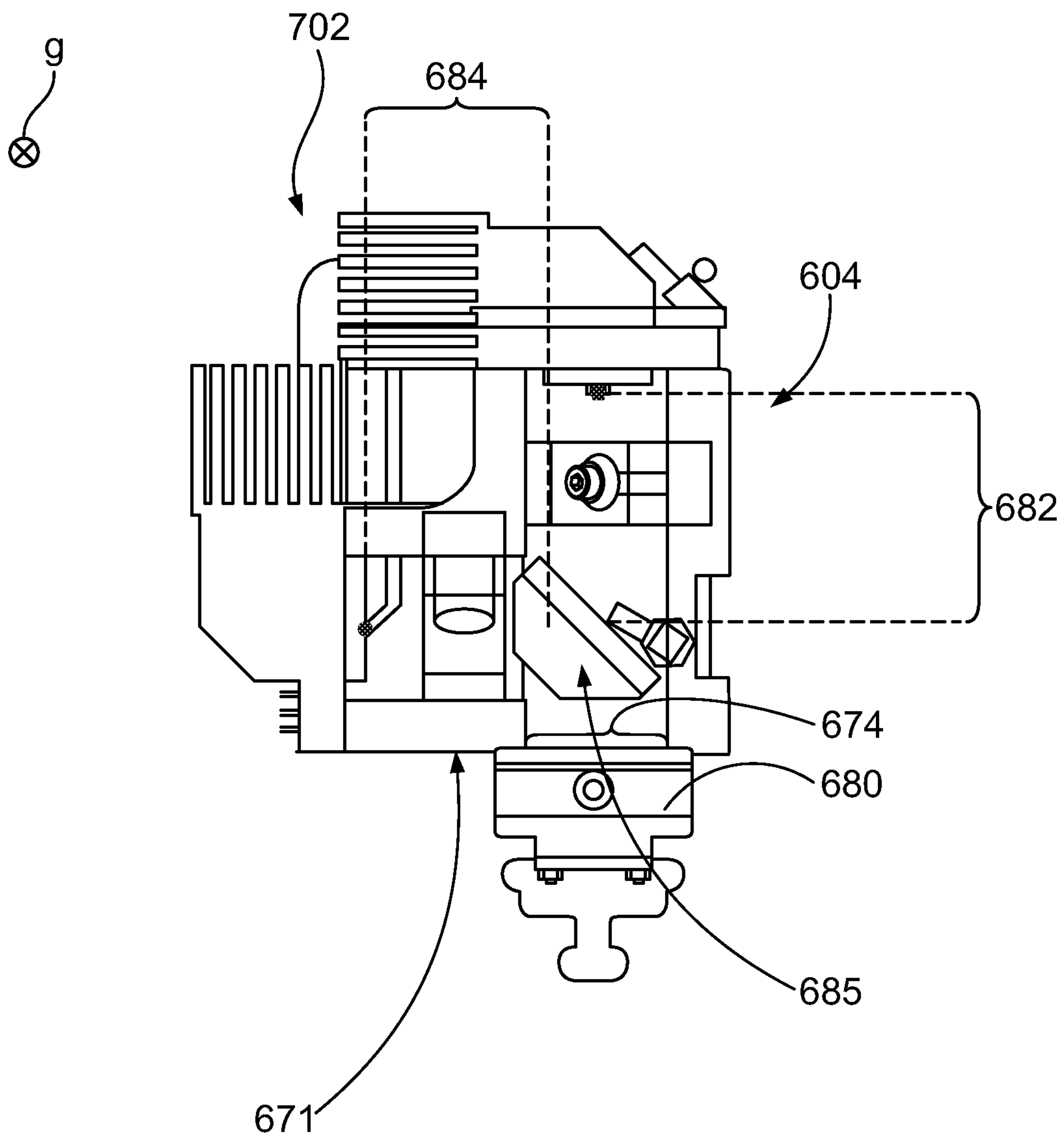


FIG. 50



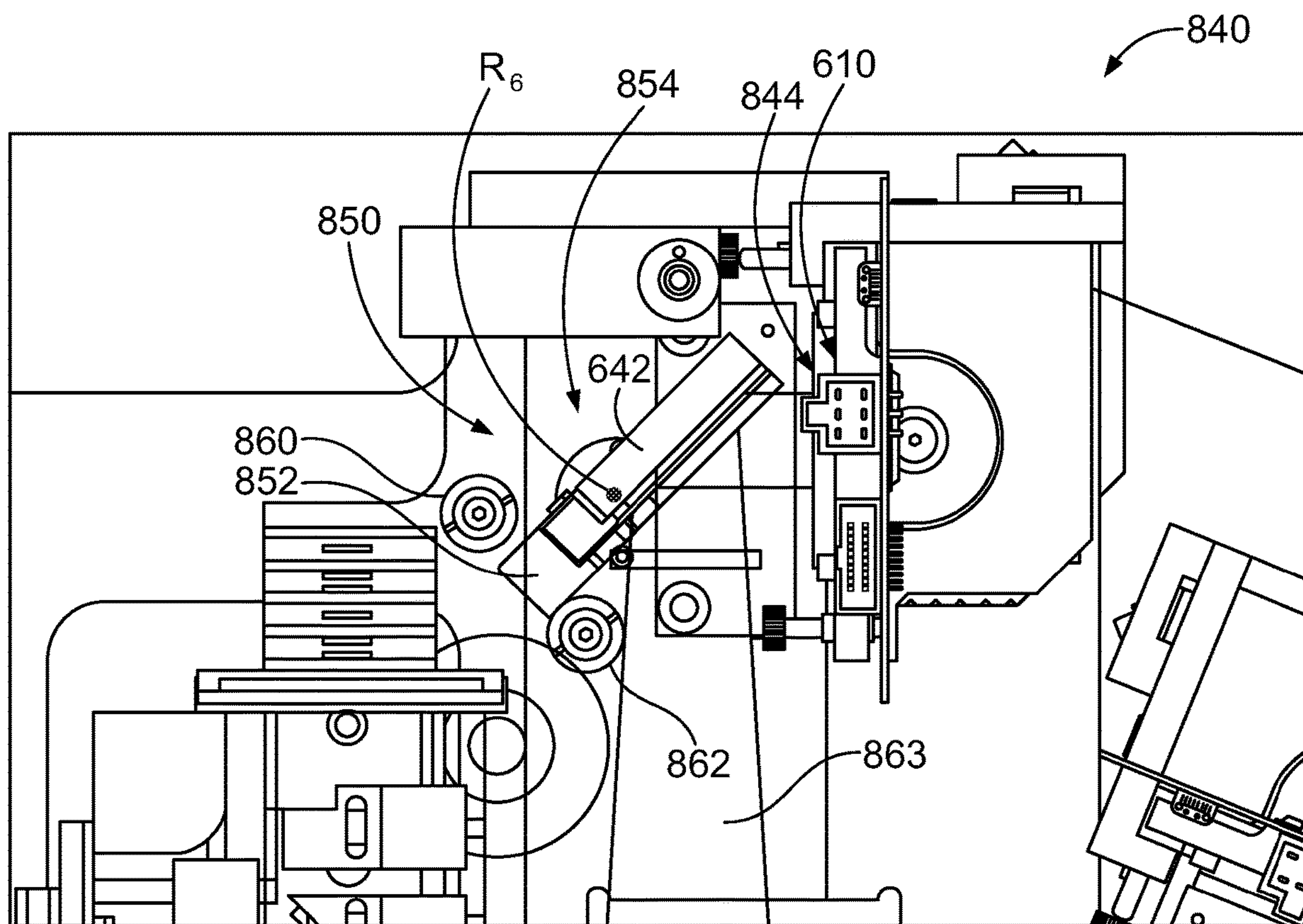


FIG. 51



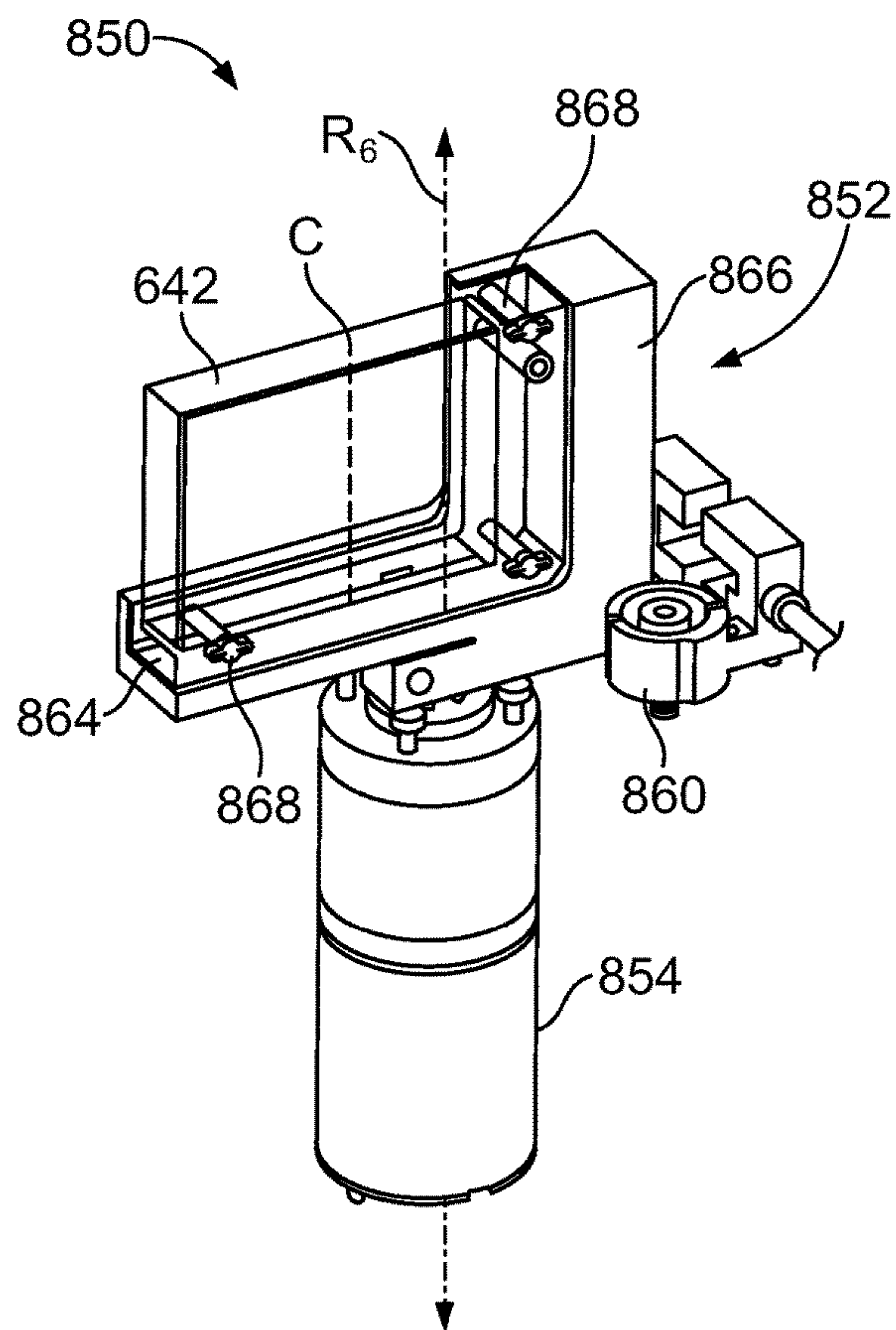


FIG. 52

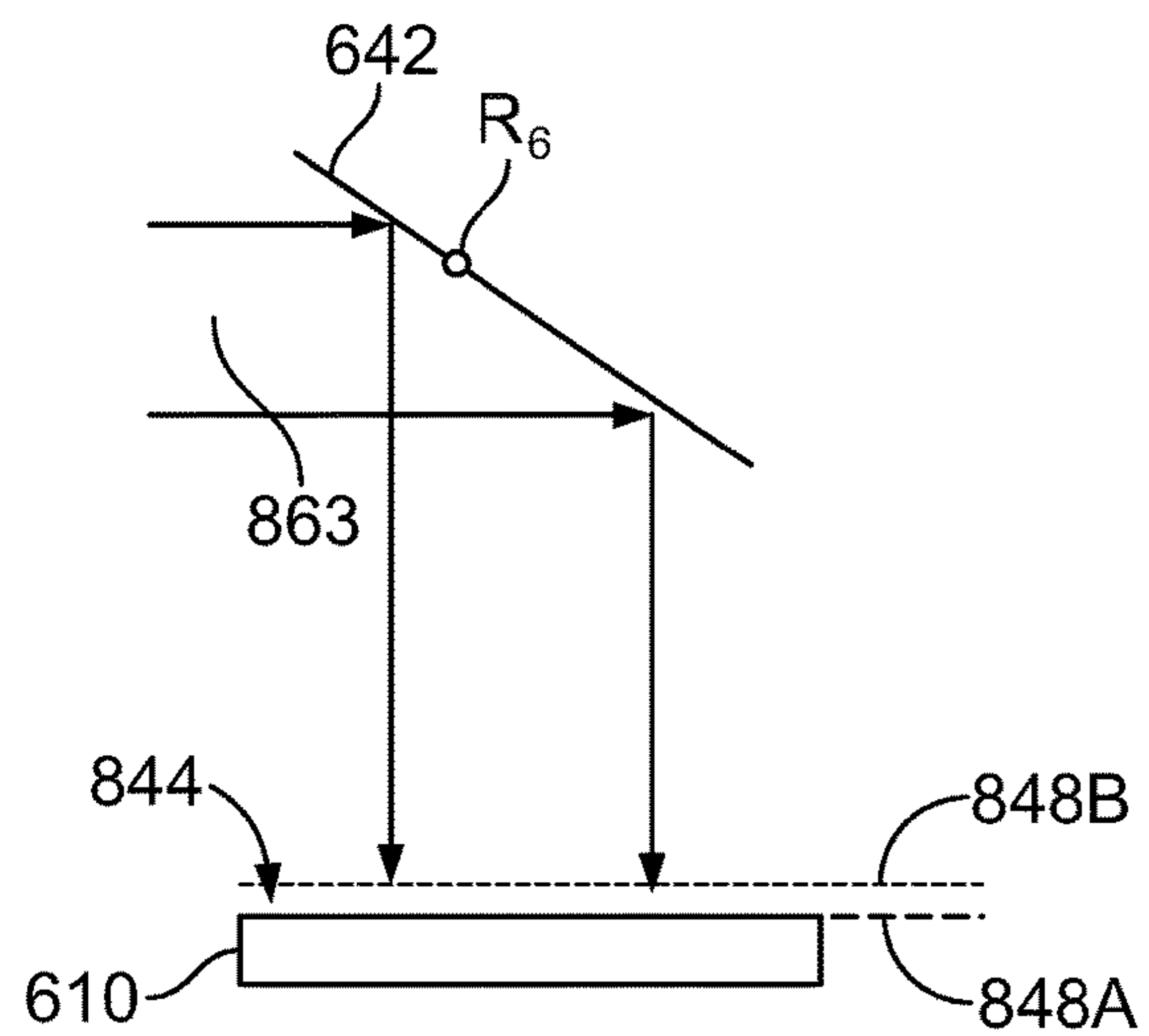


FIG. 53

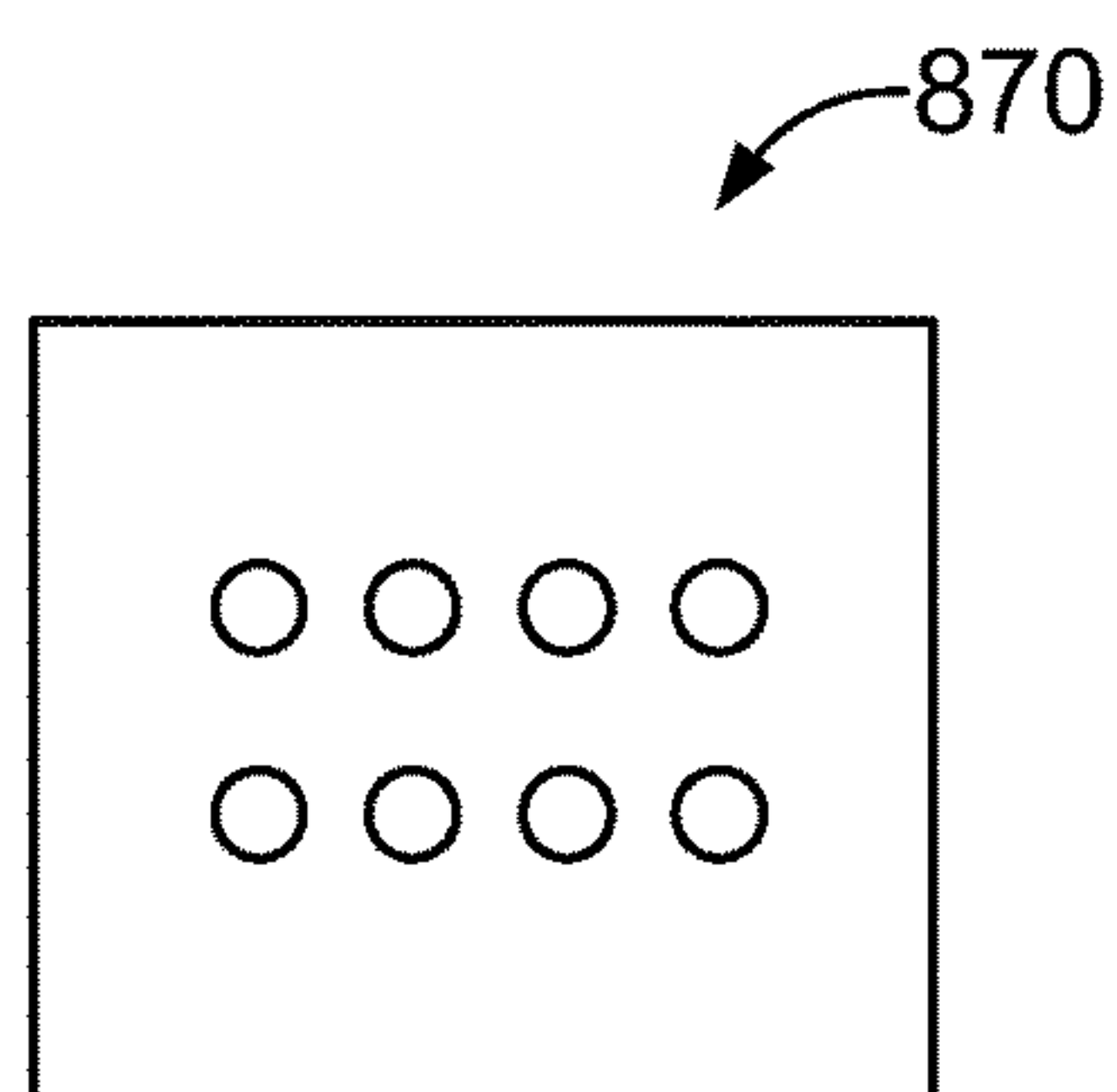


FIG. 54

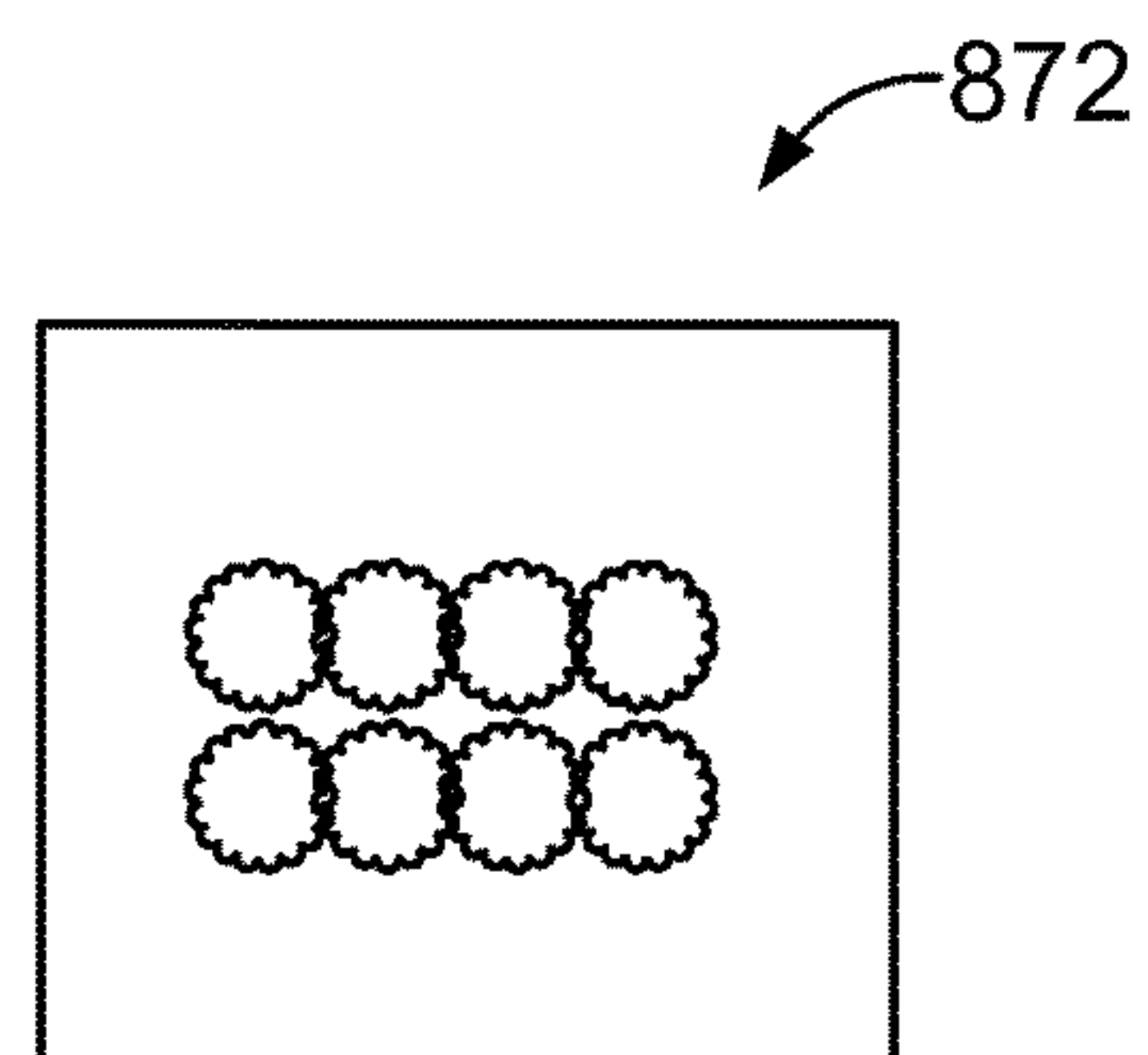


FIG. 55



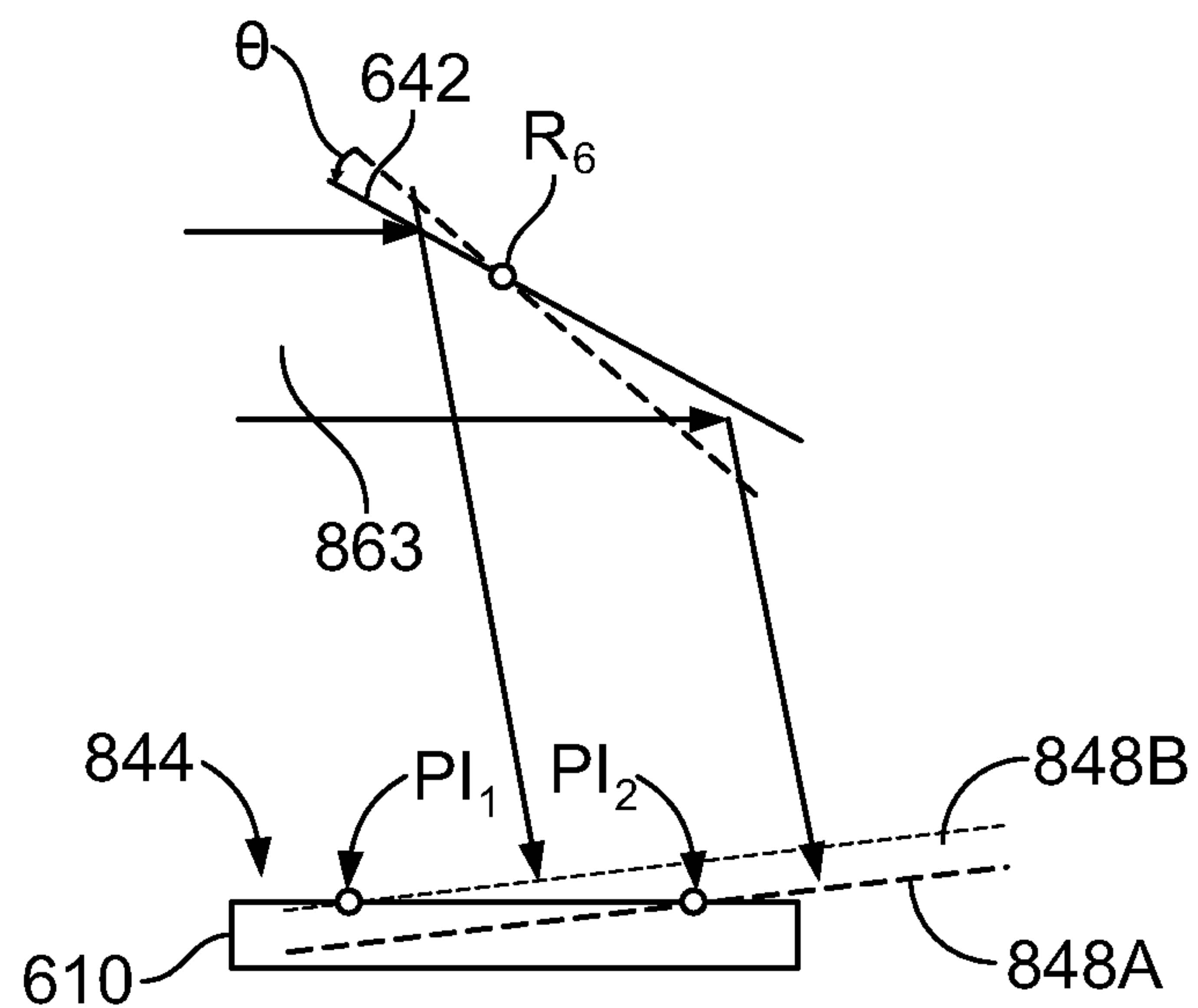


FIG. 56

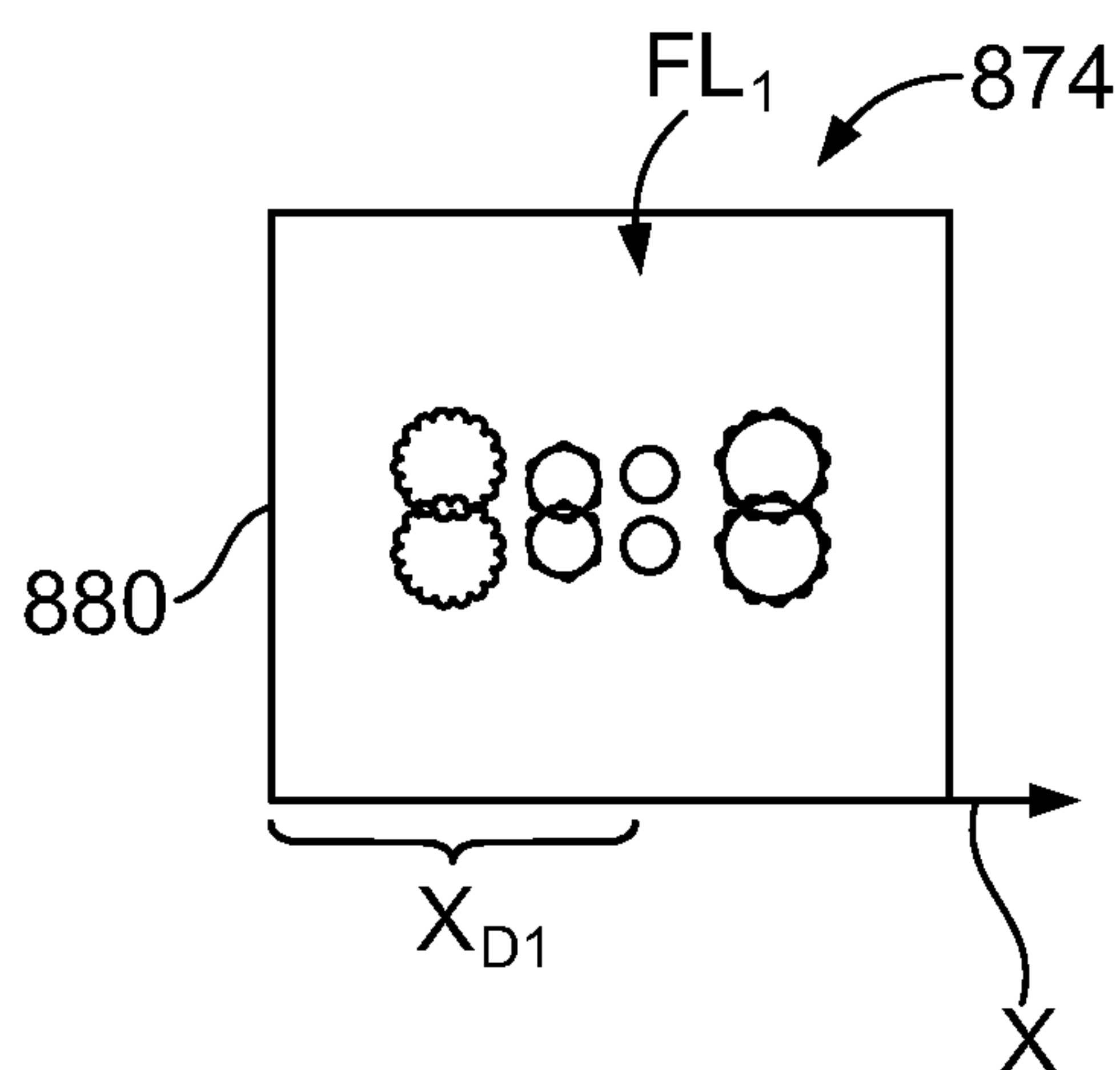


FIG. 57

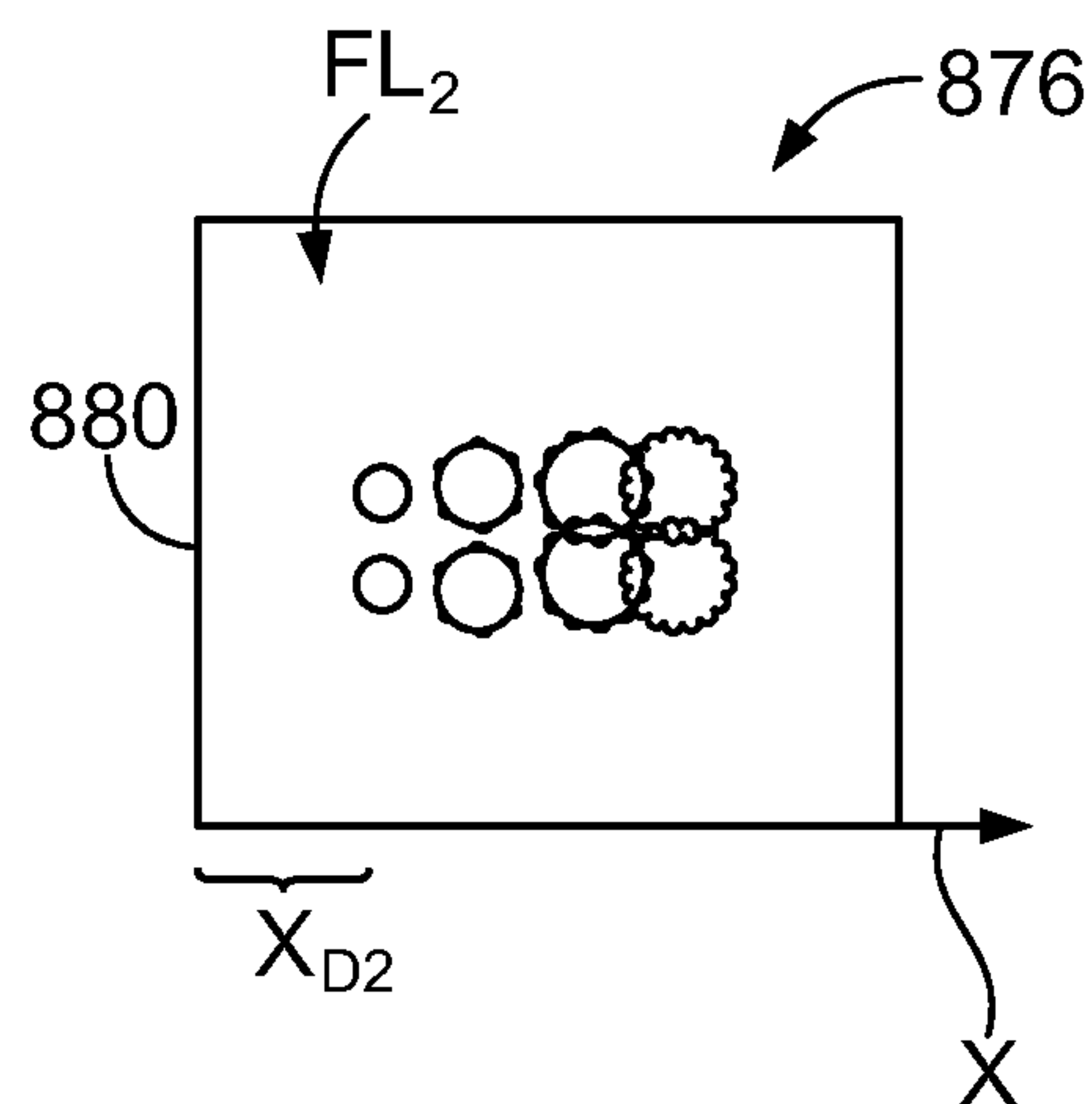
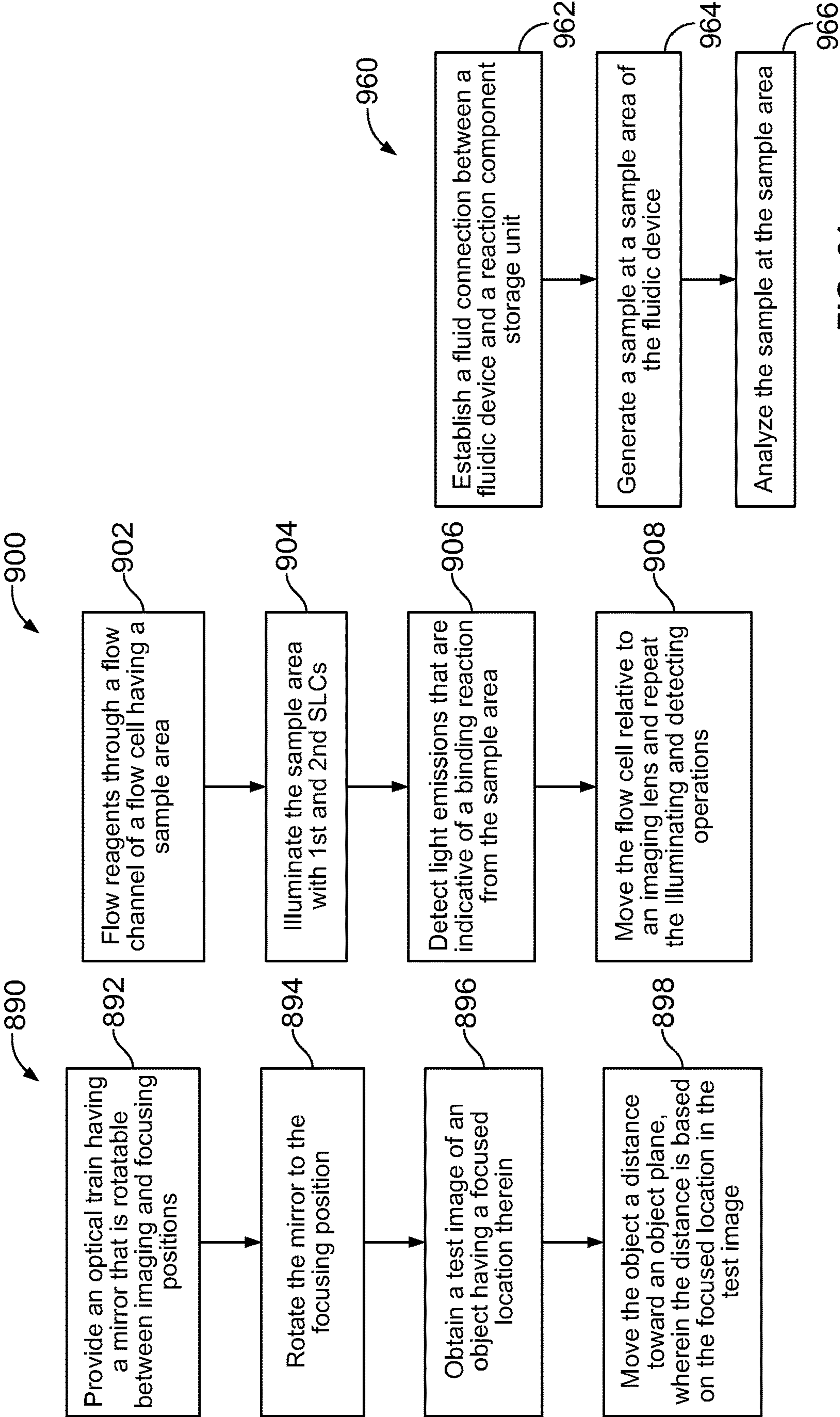


FIG. 58







## US 11,697,116 B2

## 1

**SYSTEMS, METHODS, AND APPARATUSES  
TO IMAGE A SAMPLE FOR BIOLOGICAL  
OR CHEMICAL ANALYSIS****CROSS REFERENCE TO RELATED  
APPLICATIONS**

The present application is a continuation of U.S. patent application Ser. No. 16/255,546, filed Jan. 23, 2019, which is a divisional of U.S. application Ser. No. 14/550956 (now U.S. Pat. No. 10,220,386), filed Nov. 22, 2014, which is a continuation of U.S. application Ser. No. 13/273,666 (Now U.S. Pat. No. 8,951,781), filed on Oct. 14, 2011, which relates to and claims the benefit of U.S. Provisional Application Nos. 61/431,425, filed on Jan. 10, 2011; U.S. Provisional Application No. 61/431,429, filed on Jan. 10, 2011; U.S. Provisional Application No. 61/431,439, filed on Jan. 11, 2011; U.S. Provisional Application No. 61/431,440, filed on Jan. 11, 2011; U.S. Provisional Application No. 61/438,486, filed on Feb. 1, 2011; U.S. Provisional Application No. 61/438,567, filed on Feb. 1, 2011; U.S. Provisional Application No. 61/438,530, filed on Feb. 1, 2011, the content of each of which is incorporated by reference herein in its entirety and for all purposes.

**BACKGROUND OF THE INVENTION**

Embodiments of the present invention relate generally to biological or chemical analysis and more particularly, to assay systems having fluidic devices, optical assemblies, and/or other apparatuses that may be used in detecting desired reactions in a sample.

Various assay protocols used for biological or chemical research are concerned with performing a large number of controlled reactions. In some cases, the controlled reactions are performed on support surfaces. The desired reactions may then be observed and analyzed to help identify properties or characteristics of the chemicals involved in the desired reaction. For example, in some protocols, a chemical moiety that includes an identifiable label (e.g., fluorescent label) may selectively bind to another chemical moiety under controlled conditions. These chemical reactions may be observed by exciting the labels with radiation and detecting light emissions from the labels. The light emissions may also be provided through other means, such as chemiluminescence.

Examples of such protocols include DNA sequencing. In one sequencing-by-synthesis (SBS) protocol, clusters of clonal amplicons are formed through bridge PCR on a surface of a flow channel. After generating the clusters of clonal amplicons, the amplicons may be “linearized” to make single stranded DNA (sstDNA). A series of reagents is flowed into the flow cell to complete a cycle of sequencing. Each sequencing cycle extends the sstDNA by a single nucleotide (e.g., A, T, G, C) having a unique fluorescent label. Each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in one cycle. After nucleotides are added to the sstDNAs clusters, an image in four channels is taken (i.e., one for each fluorescent label). After imaging, the fluorescent label and the terminator are chemically cleaved from the sstDNA and the growing DNA strand is ready for another cycle. Several cycles of reagent delivery and optical detection can be repeated to determine the sequences of the clonal amplicons.

However, systems configured to perform such protocols may have limited capabilities and may not be cost-effective. Thus, there is a general need for improved systems, meth-

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ods, and apparatuses that are capable of performing or being used during assay protocols, such as the SBS protocol described above, in a cost-effective, simpler, or otherwise improved manner.

**BRIEF DESCRIPTION OF THE INVENTION**

In accordance with one embodiment, a fluidic device for analyzing samples is provided. The fluidic device includes a flow cell having inlet and outlet ports and a flow channel extending therebetween. The flow cell is configured to hold a sample-of-interest. The fluidic device also includes a housing having a reception space that is configured to receive the flow cell. The reception space is sized and shaped to permit the flow cell to float relative to the housing. The fluidic device also includes a gasket that is coupled to the housing. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is positioned relative to the reception space so that the inlet and outlet ports of the flow cell are approximately aligned with the inlet and outlet passages of the gasket, respectively.

In another embodiment, a removable cartridge configured to hold and facilitate positioning a flow cell for imaging is provided. The cartridge includes a removable housing that has a reception space configured to hold the flow cell substantially within an object plane. The housing includes a pair of housing sides that face in opposite directions. The reception space extends along at least one of the housing sides so that the flow cell is exposed to an exterior of the housing through said at least one of the housing sides. The cartridge also includes a cover member that is coupled to the housing and includes a gasket. The gasket has inlet and outlet passages and comprises a compressible material. The gasket is configured to be mounted over an exposed portion of the flow cell when the flow cell is held by the housing.

In yet another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes positioning a removable fluidic device on a support surface of an imaging system. The device has a reception space, a flow cell located within the reception space, and a gasket. The flow cell extends along an object plane in the reception space and is floatable relative to the gasket within the object plane. The method also includes moving the flow cell within the reception space while on the support surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket.

In another embodiment, a method of positioning a fluidic device for sample analysis is provided. The method includes providing a fluidic device having a housing that includes a reception space and a floatable flow cell located within the reception space. The housing has recesses that are located immediately adjacent to the reception space. The method also includes positioning the fluidic device on a support structure having alignment members. The alignment members are inserted through corresponding recesses. The method also includes moving the flow cell within the reception space. The alignment members engage edges of the flow cell when the flow cell is moved within the reception space.

In another embodiment, a fluidic device holder is provided that is configured to orient a sample area with respect to mutually perpendicular X, Y, and Z-axes. The device holder includes a support structure that is configured to receive a fluidic device. The support structure includes a base surface that faces in a direction along the Z-axis and is configured to have the device positioned thereon. The device holder also includes a plurality of reference surfaces in



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respective directions along an XY-plane and an alignment assembly that includes an actuator and a movable locator arm that is operatively coupled to the actuator. The locator arm has an engagement end. The actuator moves the locator arm between retracted and biased positions to move the engagement end toward and away from the reference surfaces. The locator arm is configured to hold the device against the reference surfaces when the locator arm is in the biased position.

In another embodiment, a fluidic device holder is provided that includes a support structure having a loading region for receiving a fluidic device. The support structure includes a base surface that partially defines the loading region and is configured to have the device positioned thereon. The device holder includes a cover assembly that is coupled to the support structure and is configured to be removably mounted over the device. The cover assembly includes a cover housing having housing legs and a bridge portion that joins the housing legs. The housing legs extend in a common direction and have a viewing space that is located therebetween. The viewing space is positioned above the loading region.

In another embodiment, a method for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes is provided. The method includes providing an alignment assembly that has a movable locator arm having an engagement end. The locator arm is movable between retracted and biased positions. The method also includes positioning a fluidic device on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. The device has a sample area. The method also includes moving the locator arm to the biased position. The locator arm presses the device against the reference surfaces such that the device is held in a fixed position.

In yet another embodiment, an optical assembly is provided that includes a base plate having a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The optical assembly also includes an optical component having an optical surface that is configured to reflect light or transmit light therethrough. The optical assembly also includes a mounting device that has a component retainer and a biasing element that is operatively coupled to the retainer. The retainer holds the optical component so that a space portion of the optical surface faces the reference surface and a path portion of the optical surface extends beyond the support side into an optical path. The biasing element provides an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, a method of assembling an optical train is provided. The method includes providing a base plate that has a support side and a component-receiving space along the support side. The component-receiving space is at least partially defined by a reference surface. The method also includes inserting an optical component into the component-receiving space. The optical component has an optical surface that is configured to reflect light or transmit light therethrough. The optical surface has a space portion that faces the reference surface and a path portion that extends beyond the support side into an optical path. The

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method also includes providing an alignment force that holds the optical surface against the reference surface. In particular embodiments, the component-receiving space is a component cavity extending a depth into the base plate from the support side of the base plate. The optical and reference surfaces can have predetermined contours that are configured to position the optical surface in a predetermined orientation.

In another embodiment, an optical imaging system is provided that includes an object holder to hold and move an object and a detector to detect optical signals from the object at a detector surface. The imaging system also includes an optical train that is configured to direct the optical signals onto the detector surface. The optical train has an object plane that is proximate to the object holder and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The imaging system also includes an image analysis module that is configured to analyze a test image detected at the detector surface when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location in the test image is indicative of a position of the object with respect to the object plane. The object holder is configured to move the object toward the object plane based on the focused location.

In another embodiment, a method for controlling focus of an optical imaging system is provided. The method includes providing an optical train that is configured to direct optical signals onto a detector surface. The optical train has an object plane that is proximate to an object and an image plane that is proximate to the detector surface. The optical train includes a mirror that is rotatable between an imaging position and a focusing position. The method also includes rotating the mirror to the focusing position and obtaining a test image of the object when the mirror is in the focusing position. The test image has an optimal degree-of-focus at a focused location in the test image. The focused location is indicative of a position of the object with respect to the object plane. The method also includes moving the object toward the object plane based on the focused location.

In another embodiment, an optical imaging system is provided that includes a sample holder configured to hold a flow cell. The flow cell includes a flow channel having a sample area. The imaging system also includes a flow system that is coupled to the flow cell and configured to direct reagents through the flow channel to the sample area. The imaging system also includes an optical train that is configured to direct excitation light onto the sample area and first and second light sources. The first and second light sources have fixed positions with respect to the optical train. The first and second light sources provide first and second optical signals, respectively, for exciting the biomolecules. The imaging system also includes a system controller that is communicatively coupled to the first and second light sources and to the flow system. The controller is configured to activate the flow system to flow the reagents to the sample area and activate the first and second light sources after a predetermined synthesis time period. The light sources can be, for example, lasers or semiconductor light sources (SLSs), such as laser diodes or light emitting diodes (LEDs).

In another embodiment, a method of performing a biological assay is provided. The method includes flowing reagents through a flow channel having a sample area. The sample area includes biomolecules that are configured to chemically react with the reagents. The method also includes illuminating the sample area with first and second light



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sources. The first and second light sources provide first and second optical signals, respectively. The biomolecules provide light emissions indicative of a binding reaction when illuminated by the first or second light sources. The method also includes detecting the light emissions from the sample area. The light sources can be, for example, lasers or semiconductor light sources (SLSs), such as a laser diodes or light emitting diodes (LEDs).

In another embodiment, a flow cell is provided that includes a first layer that has a mounting surface and an outer surface that face in opposite directions and that define a thickness therebetween. The flow cell also includes a second layer having a channel surface and an outer surface that face in opposite directions and that define a thickness therebetween. The second layer has a grooved portion that extends along the channel surface. The channel surface of the second layer is secured to the mounting surface. The flow cell also includes a flow channel that is defined by the grooved portion of the channel surface and a planar section of the mounting surface. The flow channel includes an imaging portion. The thickness of the second layer is substantially uniform along the imaging portion and is configured to transmit optical signals therethrough. The thickness of the first layer is substantially uniform along the imaging portion and is configured to permit uniform transfer of thermal energy therethrough.

In another embodiment, a light source module is provided that includes a module frame having a light passage and a light source that is secured to the module frame and oriented to direct optical signals through the light passage along an optical path. The light source module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the light source. The optical component is located within the light passage such that the optical component is within the optical path.

In another embodiment, an excitation light module is provided that includes a module frame and first and second semiconductor light sources (SLSs) that are secured to the module frame. The first and second SLSs have fixed positions with respect to each other. The first and second SLSs are configured to provide different excitation optical signals. The excitation light module also includes an optical component that is secured to the module frame and has a fixed position and predetermined orientation with respect to the first and second SLSs. The optical component permits the optical signals from the first SLS to transmit therethrough and reflects the optical signals from the second SLS. The reflected and transmitted optical signals are directed along a common path out of the module frame.

In one embodiment, a method of performing a biological or chemical assay is provided. The method includes establishing a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays. The reaction components include sample-generation components and sample-analysis components. The method also includes generating a sample at the sample area of the fluidic device. The generating operation includes flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. The method also includes analyzing the sample at the sample area. The analyzing operation includes flowing at least one sample-analysis component to the sample area. Said at least one sample-analysis component reacts with the sample to provide optically detectable signals indicative of an event-of-

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interest. The generating and analyzing operations are conducted in an automated manner by the assay system.

In another embodiment, an assay system is provided that includes a fluidic device holder that is configured to hold a fluidic device and establish a fluid connection with the fluidic device. The assay system also includes a fluidic network that is configured to fluidically connect the fluidic device to a reaction component storage unit. The assay system also includes a fluidic control system that is configured to selectively flow fluids from the storage unit through the fluidic device. Furthermore, the assay system includes a system controller that has a fluidic control module. The fluidic control module is configured to instruct the fluidic control system to (a) flow different sample-generation components from the storage unit to the sample area and control reaction conditions at the sample area to generate a sample; and (b) flow at least one sample-analysis component from the storage unit to the sample area. Said at least one sample-analysis component is configured to react with the sample to provide optically detectable signals indicative of an event-of-interest. The assay system also includes an imaging system that is configured to detect the optically detectable signals from the sample. The system controller is configured to automatically generate the sample and analyze the sample by selectively controlling the fluidic device holder, the fluidic control system, and the imaging system.

In another embodiment, a method of performing a biological or chemical assay is provided. The method includes: (a) providing a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components for conducting one or more assays, the reaction components including sample-generation components and sample-analysis components; (b) flowing sample generation components according to a predetermined protocol to generate a sample at the sample area; (c) selectively controlling reaction conditions at the sample area to facilitate generating the sample; (d) flowing sample-analysis components to the sample area; and (e) detecting optical signals emitted from the sample area, the optical signals being indicative of an event-of-interest between the sample-analysis components and the sample; wherein (b)-(e) are conducted in an automated manner.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a block diagram of an assay system for performing biological or chemical assays formed in accordance with one embodiment.

FIG. 2 is a side view of a workstation configured to perform biological or chemical assays in accordance with one embodiment.

FIG. 3 is a front view of the workstation of FIG. 2.

FIG. 4 is a diagram of a fluidic network formed in accordance with one embodiment.

FIG. 5 is a perspective view of a flow cell formed in accordance with one embodiment.

FIG. 6 is a cross-section of the flow cell shown in FIG. 5 taken along the line 6-6 in FIG. 5.

FIG. 7 is a plan view of the flow cell of FIG. 5.

FIG. 8 is an enlarged view of a curved segment of a flow channel.

FIG. 9 is a perspective view of a fluidic device formed in accordance with one embodiment.

FIG. 10 is another perspective view of the fluidic device of FIG. 9.

FIG. 11 is a cross-section of the fluidic device of FIG. 9 taken along the lines 11-11 in FIG. 9.



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FIG. 12 is a perspective view of a fluidic device formed in accordance with another embodiment.

FIG. 13 is a perspective view of the fluidic device of FIG. 12.

FIG. 14 is a plan view of a fluidic device formed in accordance with one embodiment.

FIG. 15 is a side perspective view of the fluidic device of FIG. 14.

FIG. 16 is a partially exploded view of a device holder formed in accordance with one embodiment.

FIG. 17 is a perspective view of the assembled holder of FIG. 16.

FIG. 18 is a perspective view of a support structure that may be used in the holder of FIG. 16.

FIG. 19 is a top plan view of the holder of FIG. 16.

FIG. 20 is a perspective view of the holder of FIG. 16 having a cover assembly in an open position.

FIG. 21 is an enlarged plan view of the holder of FIG. 16.

FIG. 22 is a perspective view of a cover assembly that may be used in the holder of FIG. 16.

FIG. 23 is a cross-section of the cover assembly taken along the line 23-23 shown in FIG. 22.

FIG. 24 is a perspective view of a flow system that may be used with the holder of FIG. 16.

FIG. 25 is a block diagram of a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 26 is a block diagram illustrating a method of positioning a fluidic device for sample analysis in accordance with one embodiment.

FIG. 27 is a block diagram illustrating a method for orienting a sample area in accordance with one embodiment.

FIG. 28 is a perspective view of a fluid storage system formed in accordance with one embodiment.

FIG. 29 is a side cross-section of the fluid storage system of FIG. 28.

FIG. 30 is a perspective view of a removal assembly that may be used with the fluid storage system of FIG. 28.

FIG. 31 is a perspective view of a reaction component tray formed in accordance with one embodiment.

FIG. 32 is a top plan view of the tray shown in FIG. 31.

FIG. 33 is a side view of the tray shown in FIG. 31.

FIG. 34 is a front view of the tray shown in FIG. 31.

FIG. 35 is a side cross-section of a component well that may be used with the tray of FIG. 31.

FIG. 36 is a bottom perspective view of the component well of FIG. 35.

FIG. 37 is a perspective view of a component well that may be used with the tray of FIG. 31.

FIG. 38 is a diagram of an optical imaging system in accordance with one embodiment.

FIG. 39 is a perspective view of a motion-control system in accordance with one embodiment.

FIG. 40 is a perspective view of components that may be used with the motion-control system of FIG. 39.

FIG. 41 is a perspective view of an optical base plate that may be used in the imaging system of FIG. 38.

FIG. 42 is a plan view of the base plate of FIG. 41.

FIG. 43 is a perspective view of an optical component formed in accordance with one embodiment that may be used in the imaging system of FIG. 38.

FIG. 44 is a cut-away perspective view of the optical component of FIG. 43.

FIG. 45 is a front view of the optical component of FIG. 43.

FIG. 46 is a side view of the optical component of FIG. 43 during a mounting operation.

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FIG. 47 is a block diagram illustrating a method of assembling an optical train in accordance with one embodiment.

FIG. 48 is a perspective view of a light source module formed in accordance with one embodiment.

FIG. 49 is a side view of the light source module of FIG. 48.

FIG. 50 is a plan view of the light source module of FIG. 48.

FIG. 51 is a plan view of an image-focusing system in accordance with one embodiment.

FIG. 52 is a perspective view of a rotatable mirror assembly that may be used in the image-focusing system of FIG. 51.

FIG. 53 is a schematic diagram of a rotatable mirror in an imaging position that may be used in the image-focusing system of FIG. 51.

FIGS. 54 and 55 illustrate sample images that may be obtained by the image-focusing system of FIG. 51.

FIG. 56 is a schematic diagram of the rotatable mirror of FIG. 53 in a focusing position.

FIGS. 57 and 58 illustrate test images that may be obtained by the image-focusing system of FIG. 51.

FIG. 59 is a block diagram illustrating a method for controlling focus of an optical imaging system.

FIG. 60 illustrates a method for performing an assay for biological or chemical analysis.

FIG. 61 illustrates a method for performing an assay for biological or chemical analysis.

## DETAILED DESCRIPTION OF THE INVENTION

Embodiments described herein include various systems, methods, assemblies, and apparatuses used to detect desired reactions in a sample for biological or chemical analysis. In some embodiments, the desired reactions provide optical signals that are detected by an optical assembly. The optical signals may be light emissions from labels or may be transmission light that has been reflected or refracted by the sample. For example, embodiments may be used to perform or facilitate performing a sequencing protocol in which ssDNA is sequenced in a flow cell. In particular embodiments, the embodiments described herein can also perform an amplification protocol to generate a sample-of-interest for sequencing.

As used herein, a “desired reaction” includes a change in at least one of a chemical, electrical, physical, and optical property or quality of a substance that is in response to a stimulus. For example, the desired reaction may be a chemical transformation, chemical change, or chemical interaction. In particular embodiments, the desired reactions are detected by an imaging system. The imaging system may include an optical assembly that directs optical signals to a sensor (e.g., CCD or CMOS). However, in other embodiments, the imaging system may detect the optical signals directly. For example, a flow cell may be mounted onto a CMOS sensor. However, the desired reactions may also be a change in electrical properties. For example, the desired reaction may be a change in ion concentration within a solution.

Exemplary reactions include, but are not limited to, chemical reactions such as reduction, oxidation, addition, elimination, rearrangement, esterification, amidation, etherification, cyclization, or substitution; binding interactions in which a first chemical binds to a second chemical; dissociation reactions in which two or more chemicals detach



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from each other; fluorescence; luminescence; chemiluminescence; and biological reactions, such as nucleic acid replication, nucleic acid amplification, nucleic acid hybridization, nucleic acid ligation, phosphorylation, enzymatic catalysis, receptor binding, or ligand binding. The desired reaction can also be addition or elimination of a proton, for example, detectable as a change in pH of a surrounding solution or environment.

The stimulus can be at least one of physical, optical, electrical, magnetic, and chemical. For example, the stimulus may be an excitation light that excites fluorophores in a substance. The stimulus may also be a change in a surrounding environment, such as a change in concentration of certain biomolecules (e.g., enzymes or ions) in a solution. The stimulus may also be an electrical current applied to a solution within a predefined volume. In addition, the stimulus may be provided by shaking, vibrating, or moving a reaction chamber where the substance is located to create a force (e.g., centripetal force). As used herein, the phrase “in response to a stimulus” is intended to be interpreted broadly and include more direct responses to a stimulus (e.g., when a fluorophore emits energy of a specific wavelength after absorbing incident excitation light) and more indirect responses to a stimulus in that the stimulus initiates a chain of events that eventually results in the response (e.g., incorporation of a base in pyrosequencing eventually resulting in chemiluminescence). The stimulus may be immediate (e.g., excitation light incident upon a fluorophore) or gradual (e.g., change in temperature of the surrounding environment).

As used herein, the phrase “activity that is indicative of a desired reaction” and variants thereof include any detectable event, property, quality, or characteristic that may be used to facilitate determining whether a desired reaction has occurred. The detected activity may be a light signal generated in fluorescence or chemiluminescence. The detected activity may also be a change in electrical properties of a solution within a predefined volume or along a predefined area. The detected activity may be a change in temperature.

Various embodiments include providing a reaction component to a sample. As used herein, a “reaction component” or “reactant” includes any substance that may be used to obtain a desired reaction. For example, reaction components include reagents, enzymes, samples, other biomolecules, and buffer solutions. The reaction components are typically delivered to a reaction site (e.g., area where sample is located) in a solution or immobilized within a reaction site. The reaction components may interact directly or indirectly with the substance of interest.

In particular embodiments, the desired reactions are detected optically through an optical assembly. The optical assembly may include an optical train of optical components that cooperate with one another to direct the optical signals to an imaging device (e.g., CCD, CMOS, or photomultiplier tubes). However, in alternative embodiments, the sample region may be positioned immediately adjacent to an activity detector that detects the desired reactions without the use of an optical train. The activity detector may be able to detect predetermined events, properties, qualities, or characteristics within a predefined volume or area. For example, an activity detector may be able to capture an image of the predefined volume or area. An activity detector may be able to detect an ion concentration within a predefined volume of a solution or along a predefined area. Exemplary activity detectors include charged-coupled devices (CCD’s) (e.g., CCD cameras); photomultiplier tubes (PMT’s); molecular characterization devices or detectors, such as those used with nanopores; microcircuit arrangements, such as those

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described in U.S. Pat. No. 7,595,883, which is incorporated herein by reference in the entirety; and CMOS-fabricated sensors having field effect transistors (FET’s), including chemically sensitive field effect transistors (chemFET), ion-sensitive field effect transistors (ISFET), and/or metal oxide semiconductor field effect transistors (MOSFET).

As used herein, the term “optical components” includes various elements that affect the propagation of optical signals. For example, the optical components may at least one of redirect, filter, shape, magnify, or concentrate the optical signals. The optical signals that may be affected include the optical signals that are upstream from the sample and the optical signals that are downstream from the sample. In a fluorescence-detection system, upstream components include those that direct excitation radiation toward the sample and downstream components include those that direct emission radiation away from the sample. Optical components may be, for example, reflectors, dichroics, beam splitters, collimators, lenses, filters, wedges, prisms, mirrors, detectors, and the like. Optical components also include bandpass filters, optical wedges, and optical devices similar to those described herein.

As used herein, the term “optical signals” or “light signals” includes electromagnetic energy capable of being detected. The term includes light emissions from labeled biological or chemical substances and also includes transmitted light that is refracted or reflected by optical substrates. Optical or light signals, including excitation radiation that is incident upon the sample and light emissions that are provided by the sample, may have one or more spectral patterns. For example, more than one type of label may be excited in an imaging session. In such cases, the different types of labels may be excited by a common excitation light source or may be excited by different excitation light sources at different times or at the same time. Each type of label may emit optical signals having a spectral pattern that is different from the spectral pattern of other labels. For example, the spectral patterns may have different emission spectra. The light emissions may be filtered to separately detect the optical signals from other emission spectra.

As used herein, when the term “different” is used with respect to light emissions (including emission spectra or other emission characteristics), the term may be interpreted broadly to include the light emissions being distinguishable or differentiable. For example, the emission spectra of the light emissions may have wavelength ranges that at least partially overlap so long as at least a portion of one emission spectrum does not completely overlap the other emission spectrum. Different emission spectra may also have the same or similar wavelength ranges, but have different intensities that are differentiable. Different optical signals can be distinguished based on different characteristics of excitation light that produces the optical signals. For example, in fluorescence resonance energy transfer (FRET) imaging, the light emissions may be the same but the cause (e.g., excitation optical signals) of the light emissions may be different. More specifically, a first excitation wavelength can be used to excite a donor fluorophore of a donor-acceptor pair such that FRET results in emission from the acceptor and excitation of the acceptor directly will also result in emission from the acceptor. As such, differentiation of the optical signals can be based on observation of an emission signal in combination with identification of the excitation wavelength used to produce the emission. Different light emissions may have other characteristics that do not overlap, such as emission anisotropy or fluorescence lifetime. Also, when the



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light emissions are filtered, the wavelength ranges of the emission spectra may be narrowed.

The optical components may have fixed positions in the optical assembly or may be selectively moveable. As used herein, when the term “selectively” is used in conjunction with “moving” and similar terms, the phrase means that the position of the optical component may be changed in a desired manner. At least one of the locations and the orientation of the optical component may be changed. For example, in particular embodiments, a rotatable mirror is selectively moved to facilitate focusing an optical imaging system.

Different elements and components described herein may be removably coupled. As used herein, when two or more elements or components are “removably coupled” (or “removably mounted,” and other like terms) the elements are readily separable without destroying the coupled components. For instance, elements can be readily separable when the elements may be separated from each other without undue effort, without the use of a tool (i.e. by hand), or without a significant amount of time spent in separating the components. By way of example, in some embodiments, an optical device may be removably mounted to an optical base plate. In addition, flow cells and fluidic devices may be removably mounted to a device holder.

Imaging sessions include a time period in which at least a portion of the sample is imaged. One sample may undergo or be subject to multiple imaging sessions. For example, one sample may be subject to two different imaging sessions in which each imaging session attempts to detect optical signals from one or more different labels. As a specific example, a first scan along at least a portion of a nucleic acid sample may detect labels associated with nucleotides A and C and a second scan along at least a portion of the sample may detect labels associated with nucleotides G and T. In sequencing embodiments, separate sessions can occur in separate cycles of a sequencing protocol. Each cycle can include one or more imaging session. In other embodiments, detecting optical signals in different imaging sessions may include scanning different samples. Different samples may be of the same type (e.g., two microarray chips) or of different types (e.g., a flow cell and a microarray chip).

During an imaging session, optical signals provided by the sample are observed. Various types of imaging may be used with embodiments described herein. For example, embodiments described herein may utilize a “step and shoot” procedure in which regions of a sample area are individually imaged. Embodiments may also be configured to perform at least one of epi-fluorescent imaging and total-internal-reflectance-fluorescence (TIRF) imaging. In other embodiments, the sample imager is a scanning time-delay integration (TDI) system. Furthermore, the imaging sessions may include “line scanning” one or more samples such that a linear focal region of light is scanned across the sample(s). Some methods of line scanning are described, for example, in U.S. Pat. No. 7,329,860 and U.S. Pat. Pub. No. 2009/0272914, each of which the complete subject matter is incorporated herein by reference in their entirety. Imaging sessions may also include moving a point focal region of light in a raster pattern across the sample(s). In alternative embodiments, imaging sessions may include detecting light emissions that are generated, without illumination, and based entirely on emission properties of a label within the sample (e.g., a radioactive or chemiluminescent component in the sample). In alternative embodiments, flow cells may be mounted onto an imager (e.g., CCD or CMOS) that detects the desired reactions.

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As used herein, the term “sample” or “sample-of-interest” includes various materials or substances of interest that undergo an imaging session where optical signals from the material or substance are observed. In particular embodiments, a sample may include biological or chemical substances of interests and, optionally, an optical substrate or support structure that supports the biological or chemical substances. As such, a sample may or may not include an optical substrate or support structure. As used herein, the term “biological or chemical substances” may include a variety of biological or chemical substances that are suitable for being imaged or examined with the optical systems described herein. For example, biological or chemical substances include biomolecules, such as nucleosides, nucleic acids, polynucleotides, oligonucleotides, proteins, enzymes, polypeptides, antibodies, antigens, ligands, receptors, polysaccharides, carbohydrates, polyphosphates, nanopores, organelles, lipid layers, cells, tissues, organisms, and biologically active chemical compound(s) such as analogs or mimetics of the aforementioned species. Other chemical substances include labels that can be used for identification, examples of which include fluorescent labels and others set forth in further detail below.

Different types of samples may include different optical substrates or support structures that affect incident light in different manners. In particular embodiments, samples to be detected can be attached to one or more surfaces of a substrate or support structure. For example, flow cells may include one or more flow channels. In flow cells, the flow channels may be separated from the surrounding environment by top and bottom layers of the flow cell. Thus, optical signals to be detected are projected from within the support structure and may transmit through multiple layers of material having different refractive indices. For example, when detecting optical signals from an inner bottom surface of a flow channel and when detecting optical signals from above the flow channel, the optical signals that are desired to be detected may propagate through a fluid having an index of refraction, through one or more layers of the flow cells having different indices of refraction, and through the ambient environment having a different index of refraction.

As used herein, a “fluidic device” is an apparatus that includes one or more flow channels that direct fluid in a predetermined manner to conduct desired reactions. The fluidic device is configured to be fluidically coupled to a fluidic network of an assay system. By way of example, a fluidic device may include flow cells or lab-on-chip devices. Flow cells generally hold a sample along a surface for imaging by an external imaging system. Lab-on-chip devices may hold the sample and perform additional functions, such as detecting the desired reaction using an integrated detector. Fluidic devices may optionally include additional components, such as housings or imagers, that are operatively coupled to the flow channels. In particular embodiments, the channels may have channel surfaces where a sample is located, and the fluidic device can include a transparent material that permits the sample to be imaged after a desired reaction occurs.

In particular embodiments, the fluidic devices have channels with microfluidic dimensions. In such channels, the surface tension and cohesive forces of the liquid flowing therethrough and the adhesive forces between the liquid and the surfaces of the channel have at least a substantial effect on the flow of the liquid. For example, a cross-sectional area (taken perpendicular to a flow direction) of a microfluidic channel may be about 10  $\mu\text{m}^2$  or less.



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In alternative embodiments, optical imaging systems described herein may be used to scan samples that include microarrays. A microarray may include a population of different probe molecules that are attached to one or more substrates such that the different probe molecules can be differentiated from each other according to relative location. An array can include different probe molecules, or populations of the probe molecules, that are each located at a different addressable location on a substrate. Alternatively, a microarray can include separate optical substrates, such as beads, each bearing a different probe molecule, or population of the probe molecules, that can be identified according to the locations of the optical substrates on a surface to which the substrates are attached or according to the locations of the substrates in a liquid. Exemplary arrays in which separate substrates are located on a surface include, without limitation, a BeadChip Array available from Illumina®, Inc. (San Diego, Calif.) or others including beads in wells such as those described in U.S. Pat. Nos. 6,266,459, 6,355,431, 6,770,441, 6,859,570, and 7,622,294; and PCT Publication No. WO 00/63437, each of which is hereby incorporated by reference. Other arrays having particles on a surface include those set forth in US 2005/0227252; WO 05/033681; and WO 04/024328, each of which is hereby incorporated by reference.

Any of a variety of microarrays known in the art can be used. A typical microarray contains sites, sometimes referred to as features, each having a population of probes. The population of probes at each site is typically homogenous having a single species of probe, but in some embodiments the populations can each be heterogeneous. Sites or features of an array are typically discrete, being separated. The separate sites can be contiguous or they can have spaces between each other. The size of the probe sites and/or spacing between the sites can vary such that arrays can be high density, medium density or lower density. High density arrays are characterized as having sites separated by less than about 15  $\mu\text{m}$ . Medium density arrays have sites separated by about 15 to 30  $\mu\text{m}$ , while low density arrays have sites separated by greater than 30  $\mu\text{m}$ . An array useful in the invention can have sites that are separated by less than 100  $\mu\text{m}$ , 50  $\mu\text{m}$ , 10  $\mu\text{m}$ , 5  $\mu\text{m}$ , 1  $\mu\text{m}$ , or 0.5  $\mu\text{m}$ . An apparatus or method of an embodiment of the invention can be used to image an array at a resolution sufficient to distinguish sites at the above densities or density ranges.

Further examples of commercially available microarrays that can be used include, for example, an Affymetrix® GeneChip® microarray or other microarray synthesized in accordance with techniques sometimes referred to as VLSIPS™ (Very Large Scale Immobilized Polymer Synthesis) technologies as described, for example, in U.S. Pat. Nos. 5,324,633; 5,744,305; 5,451,683; 5,482,867; 5,491,074; 5,624,711; 5,795,716; 5,831,070; 5,856,101; 5,858,659; 5,874,219; 5,968,740; 5,974,164; 5,981,185; 5,981,956; 6,025,601; 6,033,860; 6,090,555; 6,136,269; 6,022,963; 6,083,697; 6,291,183; 6,309,831; 6,416,949; 6,428,752 and 6,482,591, each of which is hereby incorporated by reference. A spotted microarray can also be used in a method according to an embodiment of the invention. An exemplary spotted microarray is a CodeLink™ Array available from Amersham Biosciences. Another microarray that is useful is one that is manufactured using inkjet printing methods such as SurePrint™ Technology available from Agilent Technologies.

The systems and methods set forth herein can be used to detect the presence of a particular target molecule in a sample contacted with the microarray. This can be deter-

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mined, for example, based on binding of a labeled target analyte to a particular probe of the microarray or due to a target-dependent modification of a particular probe to incorporate, remove, or alter a label at the probe location. Any one of several assays can be used to identify or characterize targets using a microarray as described, for example, in U.S. Patent Application Publication Nos. 2003/0108867; 2003/0108900; 2003/0170684; 2003/0207295; or 2005/0181394, each of which is hereby incorporated by reference.

Furthermore, optical systems described herein may be constructed to include various components and assemblies as described in PCT application PCT/US07/07991, entitled "System and Devices for Sequence by Synthesis Analysis", filed Mar. 30, 2007 and/or to include various components and assemblies as described in International Publication No. WO 2009/042862, entitled "Fluorescence Excitation and Detection System and Method", filed Sep. 26, 2008, both of which the complete subject matter are incorporated herein by reference in their entirety. In particular embodiments, optical systems can include various components and assemblies as described in U.S. Pat. No. 7,329,860 and WO 2009/137435, of which the complete subject matter is incorporated herein by reference in their entirety. Optical systems can also include various components and assemblies as described in U.S. patent application Ser. No. 12/638,770, filed on Dec. 15, 2009, of which the complete subject matter is incorporated herein by reference in its entirety.

In particular embodiments, methods, and optical systems described herein may be used for sequencing nucleic acids. For example, sequencing-by-synthesis (SBS) protocols are particularly applicable. In SBS, a plurality of fluorescently labeled modified nucleotides are used to sequence a plurality of clusters of amplified DNA (possibly millions of clusters) present on the surface of an optical substrate (e.g., a surface that at least partially defines a channel in a flow cell). The flow cells may contain nucleic acid samples for sequencing where the flow cells are placed within the appropriate flow cell holders. The samples for sequencing can take the form of single nucleic acid molecules that are separated from each other so as to be individually resolvable, amplified populations of nucleic acid molecules in the form of clusters or other features, or beads that are attached to one or more molecules of nucleic acid. Accordingly, sequencing can be carried out on an array such as those set forth previously herein. The nucleic acids can be prepared such that they comprise an oligonucleotide primer adjacent to an unknown target sequence. To initiate the first SBS sequencing cycle, one or more differently labeled nucleotides, and DNA polymerase, etc., can be flowed into/through the flow cell by a fluid flow subsystem (not shown). Either a single type of nucleotide can be added at a time, or the nucleotides used in the sequencing procedure can be specially designed to possess a reversible termination property, thus allowing each cycle of the sequencing reaction to occur simultaneously in the presence of several types of labeled nucleotides (e.g. A, C, T, G). The nucleotides can include detectable label moieties such as fluorophores. Where the four nucleotides are mixed together, the polymerase is able to select the correct base to incorporate and each sequence is extended by a single base. Nonincorporated nucleotides can be washed away by flowing a wash solution through the flow cell. One or more lasers may excite the nucleic acids and induce fluorescence. The fluorescence emitted from the nucleic acids is based upon the fluorophores of the incorporated base, and different fluorophores may emit different wavelengths of emission light. A deblocking reagent can be added to the flow cell to remove reversible terminator groups from



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the DNA strands that were extended and detected. The deblocking reagent can then be washed away by flowing a wash solution through the flow cell. The flow cell is then ready for a further cycle of sequencing starting with introduction of a labeled nucleotide as set forth above. The fluidic and detection steps can be repeated several times to complete a sequencing run. Exemplary sequencing methods are described, for example, in Bentley et al., *Nature* 456:53-59 (2008), WO 04/018497; U.S. Pat. No. 7,057,026; WO 91/06678; WO 07/123744; U.S. Pat. Nos. 7,329,492; 7,211,414; 7,315,019; 7,405,281, and US 2008/0108082, each of which is incorporated herein by reference.

In some embodiments, nucleic acids can be attached to a surface and amplified prior to or during sequencing. For example, amplification can be carried out using bridge amplification to form nucleic acid clusters on a surface. Useful bridge amplification methods are described, for example, in U.S. Pat. No. 5,641,658; U.S. Patent Publ. No. 2002/0055100; U.S. Pat. No. 7,115,400; U.S. Patent Publ. No. 2004/0096853; U.S. Patent Publ. No. 2004/0002090; U.S. Patent Publ. No. 2007/0128624; and U.S. Patent Publ. No. 2008/0009420. Another useful method for amplifying nucleic acids on a surface is rolling circle amplification (RCA), for example, as described in Lizardi et al., *Nat. Genet.* 19:225-232 (1998) and US 2007/0099208 A1, each of which is incorporated herein by reference. Emulsion PCR on beads can also be used, for example as described in Dressman et al., *Proc. Natl. Acad. Sci. USA* 100:8817-8822 (2003), WO 05/010145, or U.S. Patent Publ. Nos. 2005/0130173 or 2005/0064460, each of which is incorporated herein by reference in its entirety.

Other sequencing techniques that are applicable for use of the methods and systems set forth herein are pyrosequencing, nanopore sequencing, and sequencing by ligation. Exemplary pyrosequencing techniques and samples that are particularly useful are described in U.S. Pat. Nos. 6,210,891; 6,258,568; 6,274,320 and Ronaghi, *Genome Research* 11:3-11 (2001), each of which is incorporated herein by reference. Exemplary nanopore techniques and samples that are also useful are described in Deamer et al., *Acc. Chem. Res.* 35:817-825 (2002); Li et al., *Nat. Mater.* 2:611-615 (2003); Soni et al., *Clin Chem.* 53:1996-2001 (2007) Healy et al., *Nanomed.* 2:459-481 (2007) and Cockroft et al., *J. Am. Chem. Soc.* 130:818-820; and U.S. Pat. No. 7,001,792, each of which is incorporated herein by reference. In particular, these methods utilize repeated steps of reagent delivery. An instrument or method set forth herein can be configured with reservoirs, valves, fluidic lines and other fluidic components along with control systems for those components in order to introduce reagents and detect optical signals according to a desired protocol such as those set forth in the references cited above. Any of a variety of samples can be used in these systems such as substrates having beads generated by emulsion PCR, substrates having zero-mode waveguides, substrates having integrated CMOS detectors, substrates having biological nanopores in lipid bilayers, solid-state substrates having synthetic nanopores, and others known in the art. Such samples are described in the context of various sequencing techniques in the references cited above and further in US 2005/0042648; US 2005/0079510; US 2005/0130173; and WO 05/010145, each of which is incorporated herein by reference.

Exemplary labels that can be detected in accordance with various embodiments, for example, when present on or within a support structure include, but are not limited to, a chromophore; luminophore; fluorophore; optically encoded nanoparticles; particles encoded with a diffraction-grating;

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electrochemiluminescent label such as Ru(bpy)<sup>32+</sup>; or moiety that can be detected based on an optical characteristic. Fluorophores that may be useful include, for example, fluorescent lanthanide complexes, including those of Europium and Terbium, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malachite green, Cy3, Cy5, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, alexa dyes, phycoerythrin, bodipy, and others known in the art such as those described in Haugland, *Molecular Probes Handbook*, (Eugene, Oreg.) 6th Edition; The Synthesgen catalog (Houston, Tex.), Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Ed., Plenum Press New York (1999), or WO 98/59066, each of which is hereby incorporated by reference. In some embodiments, the one pair of labels may be excitable by a first excitation wavelength and another pair of labels may be excitable by a second excitation wavelength.

Although embodiments are exemplified with regard to detection of samples that include biological or chemical substances supported by an optical substrate, it will be understood that other samples can be imaged by the embodiments described herein. Other exemplary samples include, but are not limited to, biological specimens such as cells or tissues, electronic chips such as those used in computer processors, and the like. Examples of some of the applications include microscopy, satellite scanners, high-resolution reprographics, fluorescent image acquisition, analyzing and sequencing of nucleic acids, DNA sequencing, sequencing-by-synthesis, imaging of microarrays, imaging of holographically encoded microparticles and the like.

FIG. 1 is a block diagram of an assay system 100 for biological or chemical analysis formed in accordance with one embodiment. In some embodiments, the assay system 100 is a workstation that may be similar to a bench-top device or desktop computer. For example, at least a majority of the systems and components for conducting the desired reactions can be within a common housing 117 of the assay system 100. In other embodiments, the assay system 100 includes one or more components, assemblies, or systems that are remotely located from the assay system 100 (e.g., a remote database). The assay system 100 may include various components, assemblies, and systems (or sub-systems) that interact with each other to perform one or more predetermined methods or assay protocols for biological or chemical analysis.

For example, the assay system 100 includes a system controller 102 that may communicate with the various components, assemblies, and systems (or sub-systems) of the assay system 100. As shown, the assay system 100 has an optical assembly 104, an excitation source assembly 106, a detector assembly 108, and a fluidic device holder 110 that supports one or more fluidic devices 112 having a sample thereon. The fluidic device may be a flow cell, such as the flow cell 200 described below, or the fluidic device 112 may be the fluidic device 300 described below.

In some embodiments, the optical assembly 104 is configured to direct incident light from the excitation source assembly 106 onto the fluidic device(s) 112. The excitation source assembly 106 may include one or more excitation light sources that are configured to excite labels associated with the sample. The excitation source assembly 106 may also be configured to provide incident light that is reflected and/or refracted by the samples. As shown, the samples may provide optical signals that include light emissions 116 and/or transmission light 118. The device holder 110 and the optical assembly 104 may be moved relative to each other. In some embodiments, the device holder 110 includes a



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motor assembly **132** that moves the fluidic device **112** with respect to the optical assembly **104**. In other embodiments, the optical assembly **104** may be moved in addition to or alternatively to the device holder **110**. The optical assembly **104** may also be configured to direct the light emissions **116** and/or transmission light **118** to the detector assembly **108**. The detector assembly **108** may include one or more imaging detectors. The imaging detectors may be, by way of example only, CCD or CMOS cameras, or photomultiplier tubes.

Also shown, the assay system **100** may include a fluidic control system **134** to control the flow of fluid throughout a fluidic network **135** (indicated by the solid lines) of the assay system **100**. The fluidic control system **134** may deliver reaction components (e.g., reagents) or other fluids to the fluidic device **112** during, for example, a sequencing protocol. The assay system **100** may also include a fluid storage system **136** that is configured to hold fluids that may be used by the assay system **100** and a temperature control system **138** that regulates the temperature of the fluid. The temperature control system **138** may also generally regulate a temperature of the assay system **100** using, for example, thermal modules, heat sinks, and blowers.

Also shown, the assay system **100** may include a user interface **140** that interacts with the user. For example, the user interface **140** may include a display **142** to display or request information from a user and a user input device **144** to receive user inputs. In some embodiments, the display **142** and the user input device **144** are the same device (e.g., touchscreen). As will be discussed in greater detail below, the assay system **100** may communicate with various components to perform the desired reactions. The assay system **100** may also be configured to analyze the detection data to provide a user with desired information.

The fluidic control system **134** is configured to direct and regulate the flow of one or more fluids through the fluidic network **135**. The fluidic control system **134** may include, for example, pumps and valves that are selectively operable for controlling fluid flow. The fluidic network **135** may be in fluid communication with the fluidic device **112** and the fluid storage system **136**. For example, select fluids may be drawn from the fluid storage system **136** and directed to the fluidic device **112** in a controlled manner, or the fluids may be drawn from the fluidic device **112** and directed toward, for example, a waste reservoir in the fluid storage system **136**. Although not shown, the fluidic control system **134** may also include flow sensors that detect a flow rate or pressure of the fluids within the fluidic network. The sensors may communicate with the system controller **102**.

The temperature control system **138** is configured to regulate the temperature of fluids at different regions of the fluidic network **135**, the fluid storage system **136**, and/or the fluidic device **112**. For example, the temperature control system **138** may include a thermocycler **113** that interfaces with the fluidic device **112** and controls the temperature of the fluid that flows along the fluidic device **112**. Although not shown, the temperature control system **138** may include sensors to detect the temperature of the fluid or other components. The sensors may communicate with the system controller **102**.

The fluid storage system **136** is in fluid communication with the fluidic device **112** and may store various reaction components or reactants that are used to conduct the desired reactions therein. The fluid storage system **136** may store fluids for washing or cleaning the fluidic network **135** or the fluidic device **112** and also for diluting the reactants. For example, the fluid storage system **136** may include various

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reservoirs to store reagents, enzymes, other biomolecules, buffer solutions, aqueous, and non-polar solutions, and the like. Furthermore, the fluid storage system **136** may also include waste reservoirs for receiving waste products.

The device holder **110** is configured to engage one or more fluidic devices **112**, for example, in at least one of a mechanical, electrical, and fluidic manner. The device holder **110** may hold the fluidic device(s) **112** in a desired orientation to facilitate the flow of fluid through the fluidic device **112** and/or imaging of the fluidic device **112**.

The system controller **102** may include any processor-based or microprocessor-based system, including systems using microcontrollers, reduced instruction set computers (RISC), application specific integrated circuits (ASICs), field programmable gate array (FPGAs), logic circuits, and any other circuit or processor capable of executing functions described herein. The above examples are exemplary only, and are thus not necessarily intended to limit the definition and/or meaning of the term system controller. In the exemplary embodiment, the system controller **102** executes a set of instructions that are stored in one or more storage elements, memories, or modules in order to at least one of obtain and analyze detection data. Storage elements may be in the form of information sources or physical memory elements within the assay system **100**.

The set of instructions may include various commands that instruct the assay system **100** to perform specific operations such as the methods and processes of the various embodiments described herein. The set of instructions may be in the form of a software program. As used herein, the terms “software” and “firmware” are interchangeable, and include any computer program stored in memory for execution by a computer, including RAM memory, ROM memory, EPROM memory, EEPROM memory, and non-volatile RAM (NVRAM) memory. The above memory types are exemplary only, and are thus not limiting as to the types of memory usable for storage of a computer program.

The software may be in various forms such as system software or application software. Further, the software may be in the form of a collection of separate programs, or a program module within a larger program or a portion of a program module. The software also may include modular programming in the form of object-oriented programming. After obtaining the detection data, the detection data may be automatically processed by the assay system **100**, processed in response to user inputs, or processed in response to a request made by another processing machine (e.g., a remote request through a communication link).

The system controller **102** may be connected to the other components or sub-systems of the assay system **100** via communication links (indicated by dashed lines). The system controller **102** may also be communicatively connected to off-site systems or servers. The communication links may be hardwired or wireless. The system controller **102** may receive user inputs or commands, from the user interface **140**. The user input device **144** may include a keyboard, mouse, a touch-screen panel, and/or a voice recognition system, and the like. Alternatively or in addition, the user input device **144** may also be the display **142**.

FIG. **1** also illustrates a block diagram of the system controller **102**. In one embodiment, the system controller **102** includes one or more processors or modules that can communicate with one another. The system controller **102** is illustrated conceptually as a collection of modules, but may be implemented utilizing any combination of dedicated hardware boards, DSPs, processors, etc. Alternatively, the system controller **102** may be implemented utilizing an



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off-the-shelf PC with a single processor or multiple processors, with the functional operations distributed between the processors. As a further option, the modules described below may be implemented utilizing a hybrid configuration in which certain modular functions are performed utilizing dedicated hardware, while the remaining modular functions are performed utilizing an off-the-shelf PC and the like. The modules also may be implemented as software modules within a processing unit.

The system controller **102** may include a plurality of modules **151-158** that communicate with a system control module **150**. The system control module **150** may communicate with the user interface **140**. Although the modules **151-158** are shown as communicating directly with the system control module **150**, the modules **151-158** may also communicate directly with each other, the user interface **140**, or the other systems. Also, the modules **151-158** may communicate with the system control module **150** through the other modules.

The plurality of modules **151-158** include system modules **151-153** that communicate with the sub-systems. The fluidic control module **151** may communicate with the fluidic control system **134** to control the valves and flow sensors of the fluidic network **135** for controlling the flow of one or more fluids through the fluidic network **135**. The fluid storage module **152** may notify the user when fluids are low or when the waste reservoir must be replaced. The fluid storage module **152** may also communicate with the temperature control module **153** so that the fluids may be stored at a desired temperature.

The plurality of modules **151-158** may also include an image analysis module **158** that receives and analyzes the detection data (e.g., image data) from the detector assembly **108**. The processed detection data may be stored for subsequent analysis or may be transmitted to the user interface **140** to display desired information to the user. Protocol modules **155-157** communicate with the system control module **150** to control the operation of the sub-systems when conducting predetermined assay protocols. The protocol modules **155-157** may include sets of instructions for instructing the assay system **100** to perform specific operations pursuant to predetermined protocols.

The protocol module **155** may be configured to issue commands for generating a sample within the fluidic device **112**. For example, the protocol module **155** may direct the fluid storage system **136** and the temperature control system **138** to generate the sample in a sample area. In one particular embodiment, the protocol module **155** may issue commands to perform bridge PCR where clusters of clonal amplicons are formed on localized areas within a channel (or lane) of a flow cell.

The protocol module **156** may be a sequencing-by-synthesis (SBS) module configured to issue various commands for performing sequencing-by-synthesis processes. In some embodiments, the SBS module **156** may also process detection data. After generating the amplicons through bridge PCR, the SBS module **156** may provide instructions to linearize or denature the amplicons to make sstDNA and to add a sequencing primer such that the sequencing primer may be hybridized to a universal sequence that flanks a region of interest. Each sequencing cycle extends the sstDNA by a single base and is accomplished by modified DNA polymerase and a mixture of four types of nucleotides delivery of which can be instructed by the SBS module **156**. The different types of nucleotides have unique fluorescent labels, and each nucleotide has a reversible terminator that allows only a single-base incorporation to occur in each

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cycle. After a single base is added to the sstDNA, the SBS module **156** may instruct a wash step to remove nonincorporated nucleotides by flowing a wash solution through the flow cell. The SBS module **156** may further instruct the excitation source assembly and detector assembly to perform an image session(s) to detect the fluorescence in each of the four channels (i.e., one for each fluorescent label). After imaging, the SBS module **156** may instruct delivery of a deblocking reagent to chemically cleave the fluorescent label and the terminator from the sstDNA. The SBS module **156** may instruct a wash step to remove the deblocking reagent and products of the deblocking reaction. Another similar sequencing cycle may follow. In such a sequencing protocol, the SBS module **156** may instruct the fluidic control system **134** to direct a flow of reagent and enzyme solutions through the fluidic device **112**.

In some embodiments, the SBS module **157** may be configured to issue various commands for performing the steps of a pyrosequencing protocol. Pyrosequencing detects the release of inorganic pyrophosphate (PPi) as particular nucleotides are incorporated into the nascent strand (Ronaghi, M. et al. (1996) "Real-time DNA sequencing using detection of pyrophosphate release." *Analytical Biochemistry* 242(1), 84-9; Ronaghi, M. (2001) "Pyrosequencing sheds light on DNA sequencing." *Genome Res.* 11(1), 3-11; Ronaghi, M. et al. (1998) "A sequencing method based on real-time pyrophosphate." *Science* 281(5375), 363; U.S. Pat. Nos. 6,210,891; 6,258,568 and 6,274,320, the disclosures of which are incorporated herein by reference in their entireties. In pyrosequencing, released PPi can be detected by being immediately converted to adenosine triphosphate (ATP) by ATP sulfurylase, and the level of ATP generated is detected via luciferase-produced photons. In this case, the fluidic device **112** may include millions of wells where each well has a single capture bead having clonally amplified sstDNA thereon. Each well may also include other smaller beads that, for example, may carry immobilized enzymes (e.g., ATP sulfurylase and luciferase) or facilitate holding the capture bead in the well. The SBS module **157** may be configured to issue commands to the fluidic control module **151** to run consecutive cycles of fluids that carry a single type of nucleotide (e.g., 1st cycle: A; 2nd cycle: G; 3rd cycle: C; 4th cycle: T; 5th cycle: A; 6th cycle: G; 7th cycle: C; 8th cycle: T; and on). When a nucleotide is incorporated into the DNA, pyrophosphate is released thereby instigating a chain reaction where a burst of light is generated. The burst of light may be detected by a sample detector of the detector assembly. Detection data may be communicated to the system control module **150**, the image analysis module **158**, and/or the SBS module **157** for processing. The detection data may be stored for later analysis or may be analyzed by the system controller **102** and an image may be sent to the user interface **140**.

In some embodiments, the user may provide user inputs through the user interface **140** to select an assay protocol to be run by the assay system **100**. In other embodiments, the assay system **100** may automatically detect the type of fluidic device **112** that has been inserted into the device holder **110** and confirm with the user the assay protocol to be run. Alternatively, the assay system **100** may offer a limited number of assay protocols that could be run with the determined type of fluidic device **112**. The user may select the desired assay protocol, and the assay system **100** may then perform the selected assay protocol based on preprogrammed instructions.

FIGS. 2 and 3 illustrate a workstation **160** formed in accordance with one embodiment that is configured for



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biological and chemical analysis of a sample. As shown, the workstation **160** is oriented with respect to mutually perpendicular X, Y, and Z-axes. In the illustrated embodiment, a gravitational force *g* extends parallel to the Z-axis. The workstation **160** may include a workstation casing **162** (or workstation housing) that is shown in phantom in FIGS. **2** and **3**. The casing **162** is configured to hold the various elements of the workstation **160**. For example, the workstation **160** may include similar elements as described above with respect to the assay system **100** (FIG. **1**). As shown, the workstation **160** has an optical deck **164** having a plurality of optical components mounted thereto. The optical components may be part of an optical assembly, such as the optical assembly **602** described with reference to FIG. **38** et al. The optical deck **164** may have a fixed position with respect to the casing **162**.

The workstation **160** may also include a sample deck **166** that is movably coupled to the optical deck **164**. The sample deck **166** may have a slidable platform **168** that supports a fluidic device thereon having a sample-of-interest. In the illustrated embodiment, the fluidic device is the fluidic device **300** that is described in greater detail below. The platform **168** is configured to slide with respect to the optical deck **166** and, more specifically, with respect to an imaging lens of the optical assembly **602**. To this end, the platform **168** may slide bi-directionally along the X-axis so that the fluidic device **300** may be positioned on the sample deck **166** and so that the imaging lens may slide over the fluidic device **300** to image the sample therein. In other embodiments, the platform **168** may be stationary and the sample deck **166** may slide bi-directionally along the X-axis to position the fluidic device **300** with respect to an imaging lens of the optical assembly **602**. Thus, the platform and sample deck can be moveable relative to each other due to movement of the sample deck, platform, or both.

Also shown, the workstation **160** may include a user interface **172**, a computing system **174** (FIG. **2**), and fluid storage units **176** and **178** (FIG. **4**). The user interface **172** may be a touchscreen that is configured to display information to a user and also receive user inputs. For example, the touchscreen may receive commands to perform predetermined assay protocols or receive inquiries from the user. The computing system **174** may include processors and modules, such as the system controller **102** and the modules **151-158** described with reference to FIG. **1**. The fluid storage units **176** and **178** may be part of a larger fluid storage system. The fluid storage unit **176** may be for collecting waste that results from performing the assays and the fluid storage unit **178** may include a buffer solution.

FIG. **4** is a diagram of a fluidic network **552** that may be used in the workstation **160** (FIG. **2**). As used herein, fluids may be liquids, gels, gases, or a mixture of thereof. Also, a fluid can be a mixture of two or more fluids. The fluidic network **552** may include a plurality of fluidic components (e.g., fluid lines, pumps, flow cells or other fluidic devices, manifolds, reservoirs) configured to have one or more fluids flowing therethrough. As shown, the fluidic network **552** includes a plurality of fluidic components **553-561** interconnected through fluid lines (indicated by the solid lines). In the illustrated embodiment, the fluidic network **552** includes a buffer solution container **553**, a reagent tray **554**, a multi-port valve **555**, a bypass valve **556**, a flow rate sensor **557**, a flow cell **558**, another flow rate sensor **559**, a pump **560**, and a waste reservoir **561**. Fluid flow directions are indicated by arrows along the fluid lines. In addition to the fluidic components **553-561**, the fluidic network may also include other fluidic components.

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The reagent tray **554** may be similar to the reaction component tray (or reaction component storage unit) **1020** described in greater detail below. The tray **1020** may include various containers (e.g., vials or tubes) containing reaction components for performing assays with embodiments described herein. Operation of the multi-port valve **555** may be controlled by an assay system, such as the assay system **100**, to selectively flow different fluids, including mixtures thereof, to the flow cell **558**. The flow cell **558** may be the flow cell **200** or the fluidic device **300**, which are described in greater detail below, or other suitable fluidic devices.

FIGS. **5-60**, which are described in greater detail below, illustrate various elements (e.g., components, devices, assemblies, systems, and the like) and methods that may be used with the workstation **160**. These elements may cooperate with one another in imaging a sample, analyzing the detection data, and providing information to a user of the workstation **160**. However, the following elements and methods may also be used independently, in other apparatuses, or with other apparatuses. For example, the flow cell **200** and the fluidic device **300** may be used in other assay systems. The optical assembly **602** (and elements thereof) may be used for examining other items, such as microcircuits. Furthermore, the device holder **400** may be used to hold other fluidic devices, such as lab-on-chip devices. Assay systems with these devices may or may not include an optical assembly to detect the desired reactions.

FIGS. **5-7** illustrate a flow cell **200** formed in accordance with one embodiment. As shown in FIGS. **5-7**, the flow cell **200** is oriented relative to the X, Y, and Z-axes. The flow cell **200** is configured to hold a sample-of-interest **205** in a flow channel **206**. The sample **205** is illustrated as a plurality of DNA clusters that can be imaged during a SBS protocol, but other samples may be used in alternative embodiments. Although only the single U-shaped flow channel **206** is illustrated, alternative embodiments may include flow cells having multiple flow channels with differently shaped paths. The flow cell **200** may be in fluid communication with a fluidic system (not shown) that is configured to deliver reagents to the sample **205** in the flow channel **206**. In some embodiments, the sample **205** may provide detectable characteristics (e.g., through fluorescence or chemiluminescence) after desired reactions occur. For instance, the flow cell **200** may have one or more sample areas or regions (i.e., areas or regions where the sample **205** is located) from which optical signals are emitted. In some embodiments, the flow cell **200** may also be used to generate the sample **205** for performing a biological or chemical assay. For example, the flow cell **200** may be used to generate the clusters of DNA before the SBS protocol is performed.

As shown in FIGS. **5-7**, the flow cell **200** can include a first layer **202** and a second layer **204** that are secured together and define the flow channel **206** therebetween. The first layer **202** has a mounting surface **208** and an outer or exterior surface **210** (FIGS. **5** and **6**). The mounting and outer surfaces **208** and **210** face in opposite directions along the Z-axis and define a thickness *T<sub>i</sub>* (FIGS. **5** and **6**) therebetween. The thickness *T<sub>i</sub>* is substantially uniform along an XY-plane, but may vary in alternative embodiments. The second layer **204** has a channel surface **212** (FIG. **6**) and an outer or exterior surface **214**. The channel and outer surfaces **212** and **214** face in opposite directions along the Z-axis and define a thickness *T<sub>2</sub>* (FIG. **6**) therebetween.

Also shown in FIG. **5**, the first layer **202** has a dimension or length *L<sub>1</sub>* measured along the X-axis and another dimension or width *W<sub>1</sub>* measured along the Y-axis. In some embodiments, the flow cell **200** may be characterized as a



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microdevice. Microdevices may be difficult to hold or move by an individual's hands. For example, the length  $L_1$  of the flow cell **200** may be about 100 mm, or about 50 mm, or less. In particular embodiments, the length  $L_1$  is about 30 mm or less. In some embodiments, the width  $W_1$  may be about 35 mm, or about 25 mm or less or, more particularly, the width  $W_1$  may be about 15 mm or less. Furthermore, a combined or total height  $H_T$  shown in FIG. 7 (e.g., a sum of thicknesses  $T_1$  and  $T_2$ ) may be about 10 mm, or about 5 mm or less. More specifically, the height  $H_T$  may be about 2 mm or about 1.5 mm or less.

The flow cell **200** includes edges **231-234** that are linear in the illustrated embodiment. Edges **231** and **233** are spaced apart by the width  $W_1$  and extend the length  $L_1$  of the flow cell **200**. Edges **232** and **234** are spaced apart by the length  $L_1$  and extend along the width  $W_1$ . Also shown, the second layer **204** may have a dimension or length  $L_2$  measured along the X-axis and another dimension or width  $W_2$  measured along the Y-axis. In the illustrated embodiment, the edges **231-234** define a perimeter of the flow cell **200** and extend along a common cell plane that extends parallel to the XY-plane. Also shown, the second layer **204** may have edges **241-244** that are similarly oriented as the edges **231-234** as shown in FIG. 5.

In the illustrated embodiment, the width  $W_1$  is substantially greater than the width  $W_2$ , and the second layer **204** is positioned on only a portion of the mounting surface **208**. As such, the mounting surface **208** includes exposed grip portions **208A** and **208B** on opposite sides of the second layer **204**. The width  $W_2$  extends between the grip portions **208A** and **208B**. The flow cell **200** may also have cell sides **256** and **258** that face in opposite directions along the Z-axis. In the illustrated embodiment, the cell side **256** includes the grip portions **208A** and **208B** and the exterior surface **214**, and the cell side **258** includes the exterior surface **210**. Also shown, the flow cell **200** may extend lengthwise between opposite first and second cell ends **246** and **248**. In the illustrated embodiment, the edges **232** and **242** are substantially flush with respect to each other at the first cell end **246**, and the edges **234** and **244** are substantially flush with respect to each other at the opposite second cell end **248**.

As shown in FIG. 6, the second layer **204** has at least one grooved portion **216** that extends along the channel surface **212**. In the illustrated embodiment, the channel surface **212** is etched to form the grooved portion **216**, but the grooved portion **216** may be formed by other processes, such as machining the channel surface **212**. To assemble the flow cell **200**, the channel surface **212** of the second layer **204** is mounted onto and secured to the mounting surface **208** of the first layer **202**. For example, the channel and mounting surfaces **212** and **208** may be bonded together using an adhesive (e.g., light-activated resin) that prevents leakage from the flow cell **200**. In other embodiments, the channel and mounting surfaces **212** and **208** may be secured together by other adhesives or mechanically interlocked and/or held together. Thus, the first layer **202** is configured to cover the grooved portion **216** of the second layer **204** to form the flow channel **206**. In the illustrated embodiment, the grooved portion **216** may be a single continuous groove that extends substantially the length  $L_2$  toward the first end, curves, and then extends substantially the length  $L_2$  toward the second end. Thus, the flow channel **206** may be substantially U-shaped.

In FIGS. 5-7 the sample **205** is shown as being located along only the mounting surface **208**. However, in other embodiments, the sample **205** may be located on any surface that defines the flow channel **206**. For instance, the sample

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**205** may also be located on the mating surface **212** of the grooved portion **216** that partially defines the flow channel **206**.

In the illustrated embodiment, the flow channel **206** may include a plurality of channel segments **250-252**. Different channel segments may have different dimensions with respect to the immediately upstream or downstream channel segment. In the illustrated embodiment, the flow channel **206** may include a channel segment **250**, which may also be referred to as the imaging segment **250**. The channel segment **250** may have a sample area that is configured to be imaged by an imaging system (not shown). The flow channel **206** may also have channel segments **251** and **252**, which may also be referred to as non-imaging segments **250** and **252**. As shown, the channel segments **250** and **252** extend parallel to each other through the flow cell **200**. The channel segments **251** and **252** of the flow channel **206** may be sized and shaped relative to the channel segment **250** to control the flow of fluid and gases that may flow therethrough.

For example, FIG. 7 also illustrates cross-sections  $C_1$ - $C_3$  of the channel segments **250-252**, respectively, that are taken perpendicular to a flow direction  $F_1$ . In some embodiments, the cross-sections  $C_1$ - $C_3$  may be differently sized (i.e., different cross-sectional areas) to control the flow of fluid through the flow channel **206**. For example, the cross-section  $C_1$  is greater in size than the cross-sections  $C_2$  and  $C_3$ . More specifically, the channel segments **250-252** of the flow channel **206** may have a substantially equal height  $H_1$  measured between the grooved portion **216** of the channel surface **212** (FIG. 6) and the mounting surface **208**. However, the channel segments **250-252** of the flow channel **206** may have different widths  $W_3$ - $W_5$ , respectively. The width  $W_3$  is greater than the widths  $W_4$  and  $W_5$ . The channel segment **251** may constitute a curved or elbow segment that fluidically joins the channel segments **250** and **252**. The cross-section  $C_3$  is smaller than the cross-sections  $C_1$  and  $C_2$ . For example, the width  $W_5$  is less than the widths  $W_3$  and  $W_4$ .

FIG. 8 is an enlarged view of the curved segment **251** and portions of the channel segments **250** and **252**. As described above, the channel segments **250** and **252** may extend parallel to each other. Within the flow channel **206**, it may be desirable to have a uniform flow across the sample area. For example, the fluid may include stream portions  $F_2$ - $F_4$ . Dimensions of the channel segments **250-252** may be configured so that the stream portions  $F_2$ - $F_4$  have substantially equal flow rates across the sample area. In such embodiments, different sections or portions of the sample **205** (FIG. 5) may have a substantially equal amount of time to react with reaction components within the fluid.

To this end, the curved segment **251** of the flow channel **206** may have a non-continuous contour that fluidically joins the channel segments **250** and **252**. For example, as shown in FIG. 8, the curved segment **251** may include a tapering portion **270**, an intermediate portion **276**, and a downstream portion **278**. As shown, the tapering portion **270** has a width  $W_{5A}$  that gradually reduces in size. More specifically, the curved segment **251** may include sidewalls **272** and **274** that extend inward toward each other at a substantially equal angle. The intermediate portion **276** curves from the tapering portion **270** to the downstream portion **278**. The intermediate portion **276** has a width  $W_{5B}$  that reduces in size and then begins to increase in size. The downstream portion **278** has a substantially uniform width  $W_{5C}$  throughout and extends along a substantially linear path from the intermediate portion **276** to the channel segment **252**. In other words, the



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sidewalls 272 and 274 may extend parallel to each other throughout the downstream portion 278.

Returning to FIG. 7, the flow cell 200 includes inlet and outlet ports 222 and 224, respectively. The inlet and outlet ports 222 and 224 are formed only through the second layer 204. However, in alternative embodiments, the inlet and outlet ports 222 and 224 may be formed through only the first layer 202 or through both layers 202 and 204. The flow channel 206 is in fluid communication with and extends between the inlet and outlet ports 222 and 224. In particular embodiments, the inlet and outlet ports 222 and 224 are located proximate to each other at the cell end 248 of the flow cell 200 (or proximate to the edges 234 and 244). For example, a spacing 282 that separates the inlet and outlet ports 222 and 224 may be approximately equal to the width  $W_3$ . More specifically, the spacing 282 may be about 3 mm, about 2 mm, or less. Furthermore, the channel segments 250 and 252 may be separated by a spacing 280. The spacing 280 may be less than the width  $W_3$  of the channel segment 250 or, more particularly, less than the width  $W_4$  of the channel segment 252. Thus, a path of the flow channel 206 may be substantially U-shaped and, in the illustrated embodiment, have a non-continuous contour along the curved segment 251.

In alternative embodiments, the flow channel 206 may have various paths such that the inlet and outlet ports 222 and 224 have different locations in the flow cell 200. For example, the flow channel may form a single lane that extends from the inlet port at one end of the flow cell to the outlet port at the opposite end of the flow cell.

With respect to FIG. 6, in some embodiments, the thickness  $T_2$  (FIG. 6) of the second layer 204 is substantially uniform along the imaging portion 250. The uniform thickness  $T_2$  along the imaging portion 250 may be configured to transmit optical signals therethrough. Furthermore, the thickness  $T_1$  of the first layer 202 is substantially uniform along the imaging portion 250 and configured to permit uniform transfer of thermal energy therethrough into the flow channel 206.

FIGS. 9-11 illustrate a fluidic device 300 formed in accordance with one embodiment. For illustrative purposes, the fluidic device 300 is oriented with respect to the mutually perpendicular X, Y, and Z-axes shown in FIGS. 9 and 10. FIGS. 9 and 10 are perspective views of the fluidic device 300. As shown in FIGS. 9 and 10, the fluidic device 300 includes a cartridge (or flow cell carrier) 302 and the flow cell 200. The cartridge 302 is configured to hold the flow cell 200 and facilitate orienting the flow cell 200 for an imaging session.

In some embodiments, the fluidic device 300 and the cartridge 302 may be removable such that the cartridge 302 may be removed from an imaging system (not shown) by an individual or machine without damage to the fluidic device 300 or cartridge 302. For example, the cartridge 302 may be configured to be repeatedly inserted and removed into the imaging system without damaging the cartridge 302 or rendering the cartridge 302 unsuitable for its intended purpose. In some embodiments, the fluidic device 300 and the cartridge 302 may be sized and shaped to be handheld by an individual. Furthermore, the fluidic device 300 and the cartridge 302 may be sized and shaped to be carried by an automated system.

As shown in FIGS. 9 and 10, the cartridge 302 may include a housing or carrier frame 304 and a cover member 306 that is coupled to the housing 304. The housing 304 has housing or carrier sides 303 and 305 that face in opposite directions along the Z-axis and have a height  $H_2$  (shown in

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FIG. 11) extending therebetween. As shown in FIG. 9, the housing 304 includes a bridge member 324 at a loading end 316 of the fluidic device 300 and a base member 326 at an opposite receiving end 318 of the fluidic device 300. The housing 304 also includes a pair of spaced apart leg extensions 328 and 330 that extend between the bridge and base members 324 and 326. The bridge member 324 extends between and joins the leg extensions 328 and 330. The bridge member 324 may include a recess 321 (shown in FIG. 10) that opens to an exterior of the fluidic device 300. As shown in FIG. 9, the leg extensions 328 and 330 may have a plurality of grip members 371-374 that are configured to grip the cell side 256 of the flow cell 200.

Also shown in FIG. 9, the fluidic device 300 may have a device window 315 that passes entirely through the cartridge 302 along the Z-axis. In the illustrated embodiment, the device window 315 is substantially framed by the bridge member 324, the cover member 306, and the leg extensions 328 and 330. The device window 315 includes a reception space 308 and a plurality of recesses 320 and 322 that are immediately adjacent to the reception space 308. The reception space 308 is configured to receive the flow cell 200. When the flow cell 200 is positioned within the reception space 308, the flow cell 200 is exposed to an exterior of the fluidic device 300 such that the flow cell 200 may be viewed or directly engaged along the housing side 303 and also the housing side 305. For example, the cell side 258 (also shown in FIG. 11) that faces in an opposite direction along the Z-axis relative to the cell side 256. The cell side 256 may be viewed by the imaging system or directly engaged by another component along the housing side 303. Likewise, the cell side 258 may be viewed by the imaging system or directly engaged by another component along the housing side 305.

With respect to FIGS. 9 and 10, the cover member 306 may include a cover body 340 and a gasket 342 that are coupled to each other. The gasket 342 includes inlet and outlet passages 346 and 344 (shown in FIG. 9) that are located proximate to one another. In the illustrated embodiment, the cover body 340 and the gasket 342 are co-molded into a unitary structure. When formed, the cover body 340 and the gasket 342 may have different compressible properties. For example, in particular embodiments, the gasket 342 may comprise a material that is more compressible than material of the cover body 340. However, in alternative embodiments, the cover body 340 and the gasket 342 may be separate parts that are coupled together (e.g., mechanically or using an adhesive). In other embodiments, the cover body 340 and the gasket 342 may be different portions or regions of a single continuous structure.

The cover member 306 may be movably coupled to the housing 304. For example, the cover member 306 may be rotatably coupled to the base member 326 of the housing 304. In such embodiments, the gasket 342 is rotatable about an axis of rotation  $R_1$  between a mounted position (shown in FIG. 9) and a disengaged position (shown in FIG. 10). In other embodiments in which the cover member 306 is movably coupled to the housing 304, the cover member 306 may be detachable from the housing 304. For example, when attached to the housing 304, the detachable cover member may be in a mounted position that is similar to the mounted position as shown in FIG. 9.

When unattached to the housing 304, the detachable cover member may be completely removed in a disengaged position.

Also shown in FIG. 10, the housing 304 may define a cartridge cavity 338 (FIG. 10) that is accessible when the



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cover member 306 is in the disengaged position. In some embodiments, an identification transmitter 336 may be positioned within the cartridge cavity 338. The identification transmitter 336 is configured to communicate information about the flow cell 200 to a reader. For example, the identification transmitter 336 may be an RFID tag. The information provided by the identification transmitter 336 may, for example, identify the sample in the flow cell 200, a lot number of the flow cell or sample, a date of manufacture, and/or the assay protocol to be performed when the flow cell 200 is inserted into the imaging system. The identification transmitter 336 may communicate other information as well.

FIG. 11 is a cross-section of the fluidic device 300 viewed along the Y-axis. In some embodiments, the reception space 308 is sized and shaped relative to the flow cell 200 so that the flow cell 200 is retained in the space, but in at least some configurations may float therein. As used herein, the term “float” and like terms includes the component being permitted to move a limited distance in at least one direction (e.g., along the X, Y, or Z-axes). For example, the flow cell 200 may be capable of shifting within the reception space 308 along the XY-plane. The flow cell 200 may also be capable of moving in a direction along the Z-axis within the reception space 308. Furthermore, the flow cell 200 can also be capable of slightly rotating within the reception space 308. In particular embodiments, the housing 304 permits the flow cell 200 to shift, move, and slightly rotate within the reception space 308 with respect to any of the X, Y, and Z-axes.

In some embodiments, the reception space 308 may also be characterized as the space that the fluidic device 300 allows the flow cell 200 to move freely within when the fluidic device 300 is holding the flow cell 200. Thus, dimensions of the reception space 308 may be based upon positions of reference surfaces of the fluidic device 300 that can directly engage the flow cell 200. The reference surfaces may be surfaces of the housing 304 or the cover member 306, including the gasket 342. For example, FIG. 11 illustrates a plurality of reference surfaces 381-387. The reference surfaces 381 and 382 of the grip members 371 and 372, respectively, and the reference surface 383 of the gasket 342 may limit movement of the flow cell 200 beyond a predetermined level when the flow cell 200 is held within the reception space 308. The reference surface 384 of the gasket 342 and the reference surface 385 of the bridge member 324 may limit movement of the flow cell 200 along the XY-plane. Furthermore, the reference surfaces 386 and 387 of the bridge member 324 and the cover member 306, respectively, may also limit movement of the flow cell 200 along the Z-axis. However, the reference surfaces 381-387 are exemplary only and the fluidic device 300 may have other reference surfaces that limit movement of the flow cell 200.

To assemble the fluidic device 300, the flow cell 200 may be loaded into the reception space 308. For example, the flow cell 200 may be advanced toward the device window 315 along the housing side 305. The edge 234 (FIG. 5) may be advanced between the grip members 372 and 373 and the gasket 342. The cell side 256 may then be rotated toward the grip members 371-374 so that the grip members 371-374 interface the cell side 256. The edge 232 (FIG. 5) may then be moved toward the bridge member 324 and, more specifically, the reference surface 385 of the bridge member 324. In some embodiments, the bridge member 324 may be deflected or bent to provide more space for positioning the cell end 246 (FIG. 5) thereon. When the flow cell 200 is loaded into the cartridge 302, the housing 304 and the cover

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member 306 may effectively grip the perimeter of the flow cell 200 such that the flow cell 200 is confined to move only within the reception space 308.

In alternative embodiments, the cell end 246 may be first inserted positioned by the bridge member 324 and then the gasket 342. In other embodiments, the flow cell 200 may approach the housing side 303. The grip members 371-374 may have tapered or beveled surfaces that permit the flow cell 200 to be snapped into position within the reception space 308.

Before, after, or during the loading of the flow cell 200, the cover member 306 may be moved to the disengaged position so that the identification transmitter 336 (FIG. 10) may be positioned with the cartridge cavity 338 (FIG. 10). When the gasket 342 is in the mounted position, the inlet and outlet passages 346 and 344 may have a predetermined location and orientation with respect to the housing 304 and the reception space 308. The gasket 342 may be mounted over the flow cell 200 along an exposed portion of the flow cell 200 (i.e., the cell side 256). The inlet and outlet passages 346 and 344 may be generally aligned with the inlet and outlet ports 224 and 222 (FIG. 5).

However, it should be noted that the illustrated fluidic device 300 is only one particular embodiment, and the fluidic device 300 may have different configurations in alternative embodiments. For example, in alternative embodiments, the flow cell 200 may not be exposed to the exterior of the fluidic device 300 along each of the housing sides 303 and 305. Instead, the flow cell 200 may be exposed to the exterior along only one of the housing sides (e.g., the housing side 303). Furthermore, in alternative embodiments, the cover member 306 may not be rotatably coupled to the housing 304. For example, the cover member 306 may be entirely detachable.

FIGS. 12-15 illustrate fluidic devices 900 and 920 formed in accordance with alternative embodiments that may also be used in assay systems, such as the assay system 100 (FIG. 1) and the workstation 160 (FIG. 2). The fluidic devices 900 and 920 may include similar features as the fluidic device 300. For example, as shown, in FIGS. 12 and 13, the fluidic device 900 may include a cartridge (or flow cell carrier) 902 and the flow cell 200. The cartridge 902 is configured to hold the flow cell 200 and facilitate orienting the flow cell 200 for an imaging session. The cartridge 902 includes a housing 904 and a cover member 906 that is movably mounted to the housing 904. The cover member 906 is in the mounted position in FIG. 12 and the disengaged position in FIG. 13.

Also shown in FIGS. 12 and 13, the fluidic device 900 may include a sealing member 910 that covers the inlet and outlet ports 222 and 224 (FIG. 13) of the flow cell 200. In some embodiments, the sealing member 910 is configured to facilitate retaining fluid within the flow channel 206 so that the sample 205 (FIG. 5) within the flow channel 206 remains in a fluid environment. However, in some embodiments, the sealing member 910 may be configured to prevent unwanted materials from entering the flow channel 206. As shown in FIGS. 12 and 13, the sealing member 910 is a single piece of tape that extends between the cell ends 246 and 248 (FIG. 13). An overhang portion 912 may extend away from the cell end 246. In alternative embodiments, the sealing member 910 may be more than one piece of tape (e.g., one piece of tape for each of the inlet and outlet ports 222 and 224) or the sealing member 910 may be other elements capable of covering the inlet and outlet ports 222 and 224. For example, the sealing member 910 could include plugs.

In some embodiments, the sealing member 910 covers the inlet and outlet ports 222 and 224 when the fluidic device



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900 is not mounted to an assay system. For example, the sealing member 910 may be used when the fluidic device 900 is being stored or transported, or when a sample is being grown or generated within the flow cell 200. In such instances, the sealing member 910 may be secured to the flow cell 200 and the housing 904 as shown in FIG. 13. More specifically, the sealing member 910 may couple to and extend along the cell side 256 and cover the inlet and outlet ports 222 and 224. The sealing member 910 may also couple to a base member 914 of the housing 904. The cover member 906 may then be moved to the mounted position as shown in FIG. 12 such that the sealing member 910 is sandwiched between the inlet and outlet ports 222 and 224 and the cover member 906. The cover member 906 may facilitate preventing the sealing member 910 from being inadvertently removed. In alternative embodiments, the sealing member 910 may cover inlet and outlet passages 916 and 918 of the cover member 906.

FIGS. 14 and 15 illustrate the fluidic device 920, which may also have similar features as the fluidic devices 300 and 900. As shown, the fluidic device 920 includes a cartridge (or flow cell carrier) 922 and the flow cell 200. The cartridge 922 includes a housing 924 and a cover member 925 that is movably mounted to the housing 924. The cover member 925 is only shown in the mounted position in FIGS. 14 and 15. The housing 924 and the cover member 925 may be similar to the housings 204 and 904 and the cover member 306 and 906 described above.

However, the housing 924 may also include fin projections 926 and 928. The fin projections 926 and 928 are sized and shaped to be gripped by an individual or robotic device, such as when the fluidic device 920 is being inserted in or removed from a device holder (not shown). In some embodiments, the fin projections 926 and 928 may prevent the cover assembly (not shown) from moving to the closed position if the fluidic device 920 is not properly positioned. The fin projections 926 and 928 may include tactile features 927 and 929 that are configured to be gripped by the individual. In the illustrated embodiment, the fin projections 926 and 928 are located at a receiving end 930 of the fluidic device 920. The cover member 925 may extend between the fin projections 926 and 928. However, the fin projections 926 and 928 may have other locations along the cartridge 902.

FIGS. 16-24 show various features of a fluidic device holder 400 formed in accordance with one embodiment. FIG. 16 is a partially exploded view of the holder 400. When assembled, the holder 400 may be used to hold the fluidic device 300 (FIG. 9) and the flow cell 200 (FIG. 5) in a desired orientation during an imaging session. Furthermore, the holder 400 may provide an interface between the fluidic device 300 and the imaging system (not shown) in which the holder 400 may be configured to direct fluids through the flow cell 200 and provide or remove thermal energy from the flow cell 200. Although the holder 400 is shown as holding the fluidic device 300, the holder 400 may be configured to hold other fluidic devices, such as lab-on-chip devices or flow cells without cartridges.

As shown in FIG. 16, the holder 400 may include a removable cover assembly 404 and a support structure 402. In some embodiments, the holder 400 may also include a plate structure 406 and a movable platform 408. The plate structure 406 is operatively coupled to the cover assembly 404 and includes an opening 410 therethrough. Likewise, the platform 408 includes an opening 412 therethrough. The support structure 402 may include a heat sink 414 and a thermal module (or thermocycler) 416 that is mounted onto the heat sink 414. The thermal module 416 includes a base

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portion 418 and a pedestal 420. When the holder 400 is assembled, the support structure 402, the platform 408, and the plate structure 406 are stacked with respect to each other. As such, the opening 412 is sized and shaped to receive the base portion 418, and the opening 410 is sized and shaped to receive the pedestal 420. When assembled, the cover assembly 404 may be operatively coupled to the plate structure 406 and the support structure 402.

FIG. 17 shows the assembled holder 400. In the illustrated embodiment, a panel 424 is positioned over the plate structure 406 (FIG. 16). As shown in FIGS. 16 and 17, the cover assembly 404 includes a cover housing 435 that is coupled to the plate structure 406. The cover housing 435 may be substantially U-shaped having a pair of spaced apart housing legs 436 and 438 that extend in a common direction. The housing legs 436 and 438 may be rotatably coupled to the plate structure 406 at joints 437 and 439. The cover housing 435 may also include a bridge portion 440 that extends between and joins the housing legs 436 and 438. In this manner, the cover assembly 404 may be configured to provide a viewing space 442 (FIG. 17). The viewing space 442 may be sized and shaped to permit an imaging lens (not shown) to move in a direction Dx (FIG. 17) along and over the flow cell 200.

In the illustrated embodiment, the cover assembly 404 is movable relative to the plate structure 406 or support structure 402 between an open position (shown in FIG. 16) and a closed position (shown in FIG. 17). In the open position, the cover assembly 404 is withdrawn or retracted to permit access to a loading region 422 (shown in FIG. 18) of the holder 400 so that the fluidic device 300 may be removed from or inserted into the loading region 422. In the closed position, the cover assembly 404 is mounted over the fluidic device 300. In particular embodiments, the cover assembly 404 establishes a fluid connection with the fluidic device 300 in the closed position and presses the flow cell 200 against the support structure 402.

As shown in FIG. 16, in some embodiments, the holder 400 includes a coupling mechanism 450 to facilitate holding the cover assembly 404 in the closed position. For example, the coupling mechanism 450 may include an operator-controlled element 452 that includes a button 453 that is coupled to a pair of latch openings 456 and 458. The coupling mechanism 450 also includes a pair of latch ends 454 and 455 that project away from a mating face 460 of the cover housing 435. The cover housing 435 may be biased into the open position by spring elements 464 and 466. When the cover assembly 404 is moved into the closed position by an individual or machine, the latch ends 454 and 455 are inserted into the latch openings 456 and 458, respectively, and grip the operator-controlled element 452. To move the cover assembly 404 into the open position, the individual or machine may actuate the button 453 by, for example, pushing the button 453 inward. Since the cover housing 435 is biased by the spring elements 464 and 466, the cover housing 435 is rotated away from the panel 424 (FIG. 17) about the joints 437 and 439.

In alternative embodiments, the coupling mechanism 450 may include other elements to facilitate holding the cover assembly 404 in the closed position. For example, the latch ends 454 and 455 may be replaced by magnetic elements or elements that form an interference fit with openings.

FIG. 18 is an isolated perspective view of thermal module 416 and the heat sink 414 of the support structure 402. The thermal module 416 may be configured to control a temperature of the flow cell 200 for predetermined periods of time. For example, the thermal module 416 may be config-



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ured to raise the temperature of the flow cell **200** so that DNA in the sample may denature. Furthermore, the thermal module **416** may be configured to remove thermal energy thereby lowering the temperature of the flow cell **200**. As shown, the pedestal **420** includes a base surface **430** that is sized and shaped to interface with the flow cell **200** (FIG. **5**). The base surface **430** faces in a direction along the Z-axis. The pedestal **420** may also include a plurality of alignment members **431-433** that are positioned around the base surface **430**. In the illustrated embodiment, the alignment members **431-433** have fixed positions with respect to the base surface **430**. The alignment members **431-433** have corresponding reference surfaces that are configured to engage the flow cell **200** and facilitate positioning the flow cell **200** for imaging. For example, the reference surfaces of the alignment members **431-433** may face in respective directions along the XY-plane and, as such, may be configured to limit movement of the flow cell **200** along the XY-plane. The support structure **402** may include at least a portion of the loading region **422**. The loading region **422** may be partially defined by the base surface **430** and the reference surfaces of the alignment members **431-433**.

FIGS. **19** and **20** illustrate an alignment assembly **470** that may be used with the holder **400** in accordance with one embodiment. FIG. **19** is a plan view of the holder **400** in which the cover housing **435** is shown in phantom to illustrate the alignment assembly **470**. FIG. **20** is a perspective view of the holder **400** in which the cover assembly **404** is in the open position. (In both FIGS. **19** and **20**, the panel **424** (FIG. **17**) has been removed for illustrative purposes.)

The fluidic device **300** is loaded into the loading region **422** in FIGS. **19** and **20**. When the fluidic device **300** is loaded, the flow cell **200** is placed onto the base surface **430** (FIG. **18**) and the alignment members **432**, **433**, and **431** are advanced through the recesses **320**, **322**, and **321** (FIGS. **9** and **10**) of the cartridge **302**. More specifically, the device window **315** (FIG. **9**) along the housing side **305** may be sized and shaped to be greater than a perimeter of the base surface **430**. As such, the cartridge **302** or housing **304** may be allowed to fall around the base surface **430**, but the flow cell **200** is prevented from falling by the base surface **430**. In this manner, the cell side **258** of the flow cell **200** may be pressed against the base surface **430** so that the thermal module **416** may control a temperature of the flow cell **200**. When the flow cell **200** is mounted on the base surface **430**, the reference surfaces **381-383** (FIG. **11**) of the cartridge **302** are pressed against the cell side **256** (FIG. **11**). At this time, a cell plane of the flow cell **200** that extends along the sample **205** may be substantially aligned with an object plane of the imaging system.

In the illustrated embodiment, when the fluidic device **300** is loaded into the loading region **422**, an identification reader of the assay system may detect information from the identification transmitter **336** (FIG. **10**). For example, the holder **400** may include an identification reader (not shown) in the plate structure **406** proximate to the identification transmitter **336**. The identification reading may occur before the cover assembly **404** is mounted onto the fluidic device **300**.

With reference to FIGS. **19** and **20**, the alignment assembly **470** includes various elements that cooperate together in orienting and positioning the flow cell **200** for imaging. For example, the alignment assembly **470** includes a movable locator arm **472** and an actuator **474** that is operatively coupled to the locator arm **472**. As shown, the actuator **474** includes a lever **476** and a pin element **478** that is coupled to the cover housing **435**. In the illustrated embodiment, the lever **476** is rotatable about an axis of rotation  $R_2$  (FIG. **19**).

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The lever **476** may be L-shaped having a first extension **480** configured to engage the pin element **478** and a second extension **482** configured to engage the locator arm **472**. The locator arm **472** is also rotatable about an axis of rotation  $R_3$  (FIG. **19**) and includes a finger **484** having an engagement end **486**. The alignment assembly **470** also includes a biasing element **490** (e.g., a coil spring) that engages the finger **484**. The engagement end **486** is configured to engage the cartridge **302** of the fluidic device **300**. In alternative embodiments, the engagement end **486** may be configured to directly engage the flow cell **200**.

The alignment assembly **470** is in an engaged arrangement in FIG. **19** and in a withdrawn arrangement in FIG. **20**. The locator arm **472** is in a retracted position when the alignment assembly **470** is in the withdrawn arrangement and in a biased position when the alignment assembly **470** is in the engaged arrangement. To align the flow cell **200** in the loading region **422**, the alignment assembly **470** is changed from the withdrawn arrangement to the engaged arrangement. For example, when the cover housing **435** is moved to the open position shown in FIG. **20**, the pin element **478** engages the first extension **480** of the lever **476** causing the lever **476** to rotate about the axis  $R_2$  in a counter-clockwise direction (as shown in FIG. **19**). The cover housing **435** may be maintained in the open position by the spring elements **464** and **466** (FIG. **16**). When the lever **476** is rotated, the second extension **482** rotates about the axis  $R_2$  and engages the locator arm **472**. The locator arm **472** is rotated about the axis  $R_3$  in a clockwise direction (as shown in FIG. **19**). When the locator arm **472** is rotated, the locator arm **472** is moved to the retracted position. When moved to the retracted position, the engagement end **486** is moved away from the reference surfaces of the alignment members **431-433**.

To change the alignment assembly **470** from the withdrawn arrangement to the engaged arrangement, the cover housing **435** may be rotated toward the fluidic device **300** and mounted over the flow cell **200**. When the cover housing **435** is moved toward the fluidic device **300**, the pin element **478** is rotated away from the first extension **480** of the lever **476**. When the second extension **482** moves away from the locator arm **472**, potential energy stored in the biasing element **490** may cause the locator arm **472** to rotate in a counter-clockwise direction such that the engagement end **486** presses against the cartridge **302**. As such, the locator arm **472** is moved to the biased position. When moved to the biased position, the engagement end **486** is moved toward the reference surfaces of the alignment members **431-433**.

FIG. **21** is an enlarged plan view of the fluidic device **300** in the loading region **422** when the engagement end **486** of the locator arm **472** is pressed against the cartridge **302**. The engagement end **486** may be configured to move within the XY-plane between the retracted and biased positions. When the engagement end **486** is moved toward the biased position and presses against the cartridge **302**, the engagement end **486** provides a force  $F_{xy}$  against the cartridge **302**. The cartridge **302** may shift along the XY-plane and/or press the flow cell **200** against the reference surfaces of the alignment members **431-433**. The force  $F_{xy}$  has an X-component and a Y-component. The X-component may press the flow cell **200** against the alignment member **431**, and the Y-component may press the flow cell **200** against the alignment members **432** and **433**. As such, the alignment member **431** may stop movement of the flow cell **200** in a direction along the X-axis, and the alignment members **432** and **433** may stop movement of the flow cell **200** in a direction along the Y-axis.



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Before the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 of the cover member 306 may be approximately aligned with the inlet and outlet ports 224 and 222 (FIG. 7), respectively, of the flow cell 200. After the alignment assembly 470 is changed to the engaged arrangement, the inlet and outlet passages 346 and 344 are effectively (or operatively) aligned with the inlet and outlet ports 224 and 222 so that fluid may effectively flow therethrough.

Accordingly, the cover assembly 404 may be operatively coupled to the alignment assembly 470 such that one step or action causes the alignment assembly 470 to engage the fluidic device 300. More specifically, as the cover assembly 404 is mounted over the device in the closed position, the actuator 474 moves the locator arm 472 to the biased position. In the biased position, the locator arm 472 holds the flow cell 200 against the reference surfaces of the alignment members 431-433 in a fixed position along the XY-plane. When the cover assembly 404 is in the closed position, the viewing space 442 (FIG. 17) may be located over the flow cell 200 so that an imaging lens may move along the flow cell 200 to image the flow channel 206. As the cover assembly 404 is moved to the open position, the actuator 474 moves the locator arm 472 to the retracted position. However, in the illustrated embodiment, the flow cell 200 remains in position when the locator arm 472 is retracted. Accordingly, the flow cell 200 may be floatable relative to various elements. For example, the flow cell 200 may be floatable with respect to the cover member 306 and the gasket 342 when the cover member 306 is in the mounted position. The flow cell 200 may also be floatable relative to the cover assembly 404 and the base surface 430.

In some embodiments, the alignment assembly 470 and the cover assembly 404 may operate at a predetermined sequence. For example, in particular embodiments, the locator arm 472 is configured to hold the flow cell 200 against the alignment members 431-433 in the fixed position before the cover assembly 404 reaches the closed position. When the cover assembly 404 reaches the closed position, the cover assembly 404 may facilitate pressing the flow cell 200 against the base surface 430 and also pressing the inlet and outlet passages 346 and 344 against the inlet and outlet ports 224 and 222. Generally, the alignment assembly 470 can be configured to position the flow cell 200 in the x and y dimensions after the base surface 430 positions the flow cell 200 in the z dimension. Alternatively, an alignment assembly can be configured to position the flow cell 200 first in the x and y dimensions and then in the z dimension. Thus, alignment in the x, y and z dimensions can occur sequentially and in various orders in response to a single step or motion carried out by a user.

In alternative embodiments, the alignment assembly 470 may not be operatively coupled to the cover assembly 404 as described above. Instead, the alignment assembly 470 and the cover assembly 404 may operate independently from each other. As such, an individual may be required to perform a plurality of steps to align the flow cell 200 and fluidically couple the flow cell 200. For example, the alignment assembly 470 can be separately actuated by an individual thereby moving the locator arm 472 to align the flow cell 200. After the flow cell 200 is aligned, the individual may then lower the cover assembly 404 onto the flow cell 200. Furthermore, the alignment assembly 470 may comprise additional and/or other components than those described above.

FIG. 22 is an isolated perspective view of the cover assembly 404 in the closed position. FIG. 22 illustrates

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dimensions of the viewing space 442. As shown, the cover housing 435 may have a top surface 492. The viewing space 442 may have a depth  $D_p$  that is measured from the top surface 492 to the fluidic device 300 or the flow cell 200. The viewing space 442 may also have a width  $W_6$  measured along the Y-axis and a length  $L_6$  measured along the X-axis. The dimensions of the viewing space 442 may be sized so that an imaging lens (not shown) may move therethrough over the flow cell 200. More specifically, an imaging lens may enter the viewing space 442 through an access opening 443 and move in a direction along the X-axis over the flow cell 200.

FIG. 23 is a cross-section of the cover assembly 404 taken along the line 23-23 in FIG. 22. In the illustrated embodiment, the cover assembly 404 may include a plurality of compression arms 494 and 496. The compression arms 494 and 496 are configured to provide respective compressive forces  $F_{C1}$  and  $F_{C2}$  against the housing side 303 of the fluidic device 300. In the illustrated embodiment, the compression arms 494 and 496 press against the cartridge 302. However, in alternative embodiments, the compression arms 494 and 496 may press against the flow cell 200.

The compressive forces  $F_{C1}$  and  $F_{C2}$  press the housing 304 of the fluidic device 300 thereby pressing the cell side 256 (FIG. 9) of the flow cell 200 against the thermal module 416. As such, the flow cell 200 may maintain intimate contact with the base surface 430 for transferring thermal energy therebetween. In the illustrated embodiment, the compression arms 494 and 496 operate independently of each other. For example, each of the compression arms 494 and 496 is operatively coupled to respective compression springs 495 and 497.

As shown in FIG. 23, the compression arms 494 and 496 extend toward the viewing space 442 and the loading region 422. The compression arms 494 and 496 may engage the housing side 303 when the cover assembly 404 is moved to the closed position. As the compression arms 494 and 496 press against the housing side 303, resistance from the housing side 303 may cause the compression arms 494 and 496 to rotate about axes  $R_4$  and  $R_5$ . Each of the compression springs 495 and 497 may resist the rotation of the respective compression arm thereby providing the corresponding compressive force  $F_C$  against the housing side 303. Accordingly, the compression arms 494 and 496 are independently biased relative to each other.

FIG. 24 is an isolated perspective view of a flow assembly 500 of the cover assembly 404 (FIG. 16). The flow assembly 500 includes a manifold body 502 and upstream and downstream flow lines 504 and 506. As shown in FIG. 16, the manifold body 502 may extend between the housing legs 436 and 438. Returning to FIG. 24, the flow lines 504 and 506 are mechanically and fluidically coupled to the manifold body 502 at body ports 508 and 510, respectively. The flow lines 504 and 506 also include line ends 514 and 516 that are configured to be inserted into the inlet and outlet passages 346 and 344 of the gasket 342.

As shown in FIG. 24, the flow assembly 500 is in a mounted position with respect to the gasket 342. In the mounted position, the line ends 514 and 516 are inserted into the inlet and outlet passages 346 and 344, respectively, so that fluid may flow through the flow cell 200. Furthermore, in the mounted position, the flow assembly 500 may press the gasket 342 (FIG. 9) against the flow cell 200 so that the fluid connection is effectively sealed. To this end, the flow assembly 500 may include biasing springs 520 and 522. The biasing springs 520 and 522 are configured to press against an interior of the cover housing 435 (FIG. 16) and provide



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a force  $F_{C3}$  against the gasket **342**. The coupling mechanism **450** (FIG. **16**) may facilitate maintaining the seal against the gasket **342**.

Accordingly, the cover assembly **404** may press against the housing **304** of the fluidic device **300** at three separate compression points. More specifically, the gasket **342** may constitute a first compression point  $P_1$  (shown in FIG. **24**) when engaged by the line ends **514** and **516**, and the compression arms **494** and **496** may contact the fluidic device **300** at second and third compression points  $P_2$  and  $P_3$  (shown in FIG. **23**). As shown in FIGS. **22-24**, the three compression points  $P_1$ - $P_3$  are distributed about the flow cell **200**. Moreover, the cover assembly **404** independently provides the compressive forces  $F_{C1}$ - $F_{C3}$  at the compression points  $P_1$ - $P_3$ . As such, the cover assembly **404** may be configured to provide a substantially uniform compressive force against the fluidic device **300** so that the flow cell **200** is uniformly pressed against the base surface **430** and the fluidic connection is sealed from leakage.

FIG. **25** is a block diagram of a method **530** of positioning a fluidic device for sample analysis. The method **530** includes positioning at **532** a removable fluidic device on a base surface. The fluidic device may be similar to the fluidic device **300** described above. For example, the fluidic device may include a reception space, a flow cell located within the reception space, and a gasket. The flow cell may extend along an object plane in the reception space and be floatable relative to the gasket within the object plane. The method **530** also includes moving the flow cell at **534** within the reception space while on the base surface so that inlet and outlet ports of the flow cell are approximately aligned with inlet and outlet passages of the gasket. The moving operation **534** may include actuating a locator arm to press the flow cell against alignment members.

FIG. **26** is a block diagram illustrating a method **540** of positioning a fluidic device for sample analysis. The fluidic device **300** may be similar to the fluidic device **300** described above. The method **540** includes providing a fluidic device at **542** having a device housing that includes a reception space and a floatable flow cell located within the reception space. The device housing may include recesses that are located immediately adjacent to the reception space. The method also includes positioning at **544** the fluidic device on a support structure having alignment members. The alignment members may be inserted through corresponding recesses. Furthermore, the method **540** may include moving the flow cell at **546** within the reception space. When the flow cell is moved within the reception space, the alignment members may engage edges of the flow cell. The moving operation **546** may include actuating a locator arm to press the flow cell against the alignment members.

FIG. **27** is a block diagram illustrating a method **550** for orienting a sample area with respect to mutually perpendicular X, Y, and Z-axes. The method **550** includes providing an alignment assembly at **552**. The alignment assembly may be similar to the alignment assembly **470** described above. More specifically, the alignment assembly may include a movable locator arm that has an engagement end. The locator arm may be movable between retracted and biased positions. The method **550** also includes positioning a fluidic device at **554** on a base surface that faces in a direction along the Z-axis and between a plurality of reference surfaces that face in respective directions along an XY-plane. Furthermore, the method **550** may include moving at **556** the locator arm to the biased position. The locator

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arm can press the device against the reference surfaces such that the device is held in a fixed position.

FIGS. **28-37** illustrate various features of a fluid storage system **1000** (FIG. **28**). The storage system **1000** is configured to store and regulate a temperature of various fluids that may be used during predetermined assays. The storage system **1000** may be used by the workstation **160** (FIG. **2**) and enclosed by the casing **162** (FIG. **3**). As shown in FIG. **28**, the storage system **1000** includes an enclosure **1002** having a base shell (or first shell) **1004** and a top shell (or second shell) **1006** that are coupled together and define a system cavity **1008** therebetween. The enclosure **1002** may also include a system door **1010** that is configured to open and provide access to the system cavity **1008**. Also shown, the storage system **1000** may include a temperature-control assembly **1012** that is coupled to a rear of the enclosure **1002** and an elevator drive motor **1014** that is located on the top shell **1006**.

FIG. **29** is a side cross-section of the storage system **1000** and illustrates the system cavity **1008** in greater detail. The storage system **1000** may also include a reaction component tray (or reaction component storage unit) **1020** and a fluid removal assembly **1022** that includes an elevator mechanism **1024**. The tray **1020** is configured to hold a plurality of tubes or containers for storing fluids. The elevator mechanism **1024** includes the drive motor **1014** and is configured to move components of the removal assembly **1022** bi-directionally along the Z-axis. In FIG. **29**, the tray **1020** is located in a fluid-removal position such that fluid held by the tray **1020** may be removed and delivered to, for example, a fluidic device for performing a desired reaction or for flushing the flow channels of the fluidic device.

Also shown, the temperature-control assembly **1012** may project into the system cavity **1008**. The temperature-control assembly **1012** is configured to control or regulate a temperature within the system cavity **1008**. In the illustrated embodiment, the temperature-control assembly **1012** includes a thermo-electric cooling (TEC) assembly.

FIG. **30** is a perspective view of the removal assembly **1022**. As shown, the removal assembly **1022** may include a pair of opposing guide rails **1032** and **1034**. The opposing guide rails **1032** and **1034** are configured to receive and direct the tray **1020** to the fluid-removal position shown in FIG. **29**. The guide rails **1032** and **1034** may include projected features or ridges **1035** that extend longitudinally along the guide rails **1032** and **1034**. The guide rails **1032** and **1034** are configured to be secured to the base shell **1004** (FIG. **28**). The removal assembly **1022** also includes support beams (or uprights) **1036** and **1038** that extend in a direction along the Z-axis. A guide plate **1040** of the removal assembly may be coupled to the support beams **1036** and **1038** at an elevated distance  $D_Z$  and project therefrom along the XY-plane. In the illustrated embodiment, the guide plate **1040** is affixed to the support beams **1036** and **1038**.

The elevator mechanism **1024** includes structural supports **1041** and **1042**, a lead screw **1044** that extends between the structural supports **1041** and **1042**, and a stage assembly **1046** that includes a transport platform **1048**. The structural supports **1041** and **1042** are secured to opposite ends of the support beams **1036** and **1038** and are configured to support the elevator mechanism **1024** during operation. Threads of the lead screw **1044** are operatively coupled to the stage assembly **1046** such that when the lead screw **1044** is rotated, the stage assembly **1046** moves in a linear direction along the Z-axis (indicated by the double arrows).

The transport platform **1048** is configured to hold an array of sipper tubes **1050**. The sipper tubes **1050** may be in fluid



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communication with a system pump (not shown) that is configured to direct a flow of fluid through the sipper tubes **1050**. As shown, the sipper tubes **1050** include distal portions **1052** that are configured to be inserted into component wells **1060** (shown in FIG. **31**) of the tray **1020**. The distal portions **1052** extend through corresponding openings **1053** of the guide plate **1040**.

The elevator mechanism **1024** is configured to move the sipper tubes **1050** between withdrawn and deposited levels. At the deposited level (shown in FIGS. **50** and **51**), the distal portions **1052** of the sipper tubes **1050** are inserted into the component wells **1060** to remove fluid therefrom. At the withdrawn level, the distal portions **1052** are completely removed from the tray **1020** such that the tray **1020** may be removed from the system cavity **1008** (FIG. **28**) without damage to the sipper tubes **1050** or the tray **1020**. More specifically, when the drive motor **1014** rotates the lead screw **1044**, the stage assembly **1046** moves along the Z-axis in a direction that is determined by a rotational direction of the lead screw **1044**. Consequently, the transport platform **1048** moves along the Z-axis while holding the sipper tubes **1050**. If the transport platform **1048** advances toward the guide plate **1040**, the distal portions **1052** slide through the corresponding openings **1053** of the guide plate **1040** toward the tray **1020**. The guide plate **1040** is configured to prevent distal portions **1052** from becoming misaligned with the component wells **1060** before the distal portions **1052** are inserted therein. When the elevator mechanism **1024** moves the stage assembly **1046** away from the guide plate **1040**, a distance ( $\Delta Z$ ) between the transport platform **1048** and the guide plate **1040** increases until the distal portions **1052** are withdrawn from the component wells **1060** of the tray **1020**.

FIG. **30** illustrates additional features for operating the elevator mechanism **1024**. For example, the stage assembly **1046** may also include a guide pin **1058** (also shown in FIG. **29**) that is affixed to and extends from the transport platform **1048** in a direction that is parallel to the sipper tubes **1050**. The guide pin **1058** also extends through a corresponding opening **1053** of the guide plate **1040**. In the illustrated embodiment, the guide pin **1058** extends a greater distance than the sipper tubes **1050** so that the guide pin **1058** reaches the tray **1020** before the sipper tubes **1050** are inserted into the component wells **1060**. Thus, if the tray **1020** is misaligned with respect to the sipper tubes **1050**, the guide pin **1058** may engage the tray **1020** and adjust the position of the tray **1020** so that the component wells **1060** are properly aligned with the corresponding sipper tubes **1050** before the sipper tubes **1050** are inserted therein.

In addition to the above, the removal assembly **1022** may include a position sensor **1062** and a location sensor (not shown). The position sensor **1062** is configured to receive a flag **1063** (shown in FIG. **34**) of the tray **1020** to determine that the tray **1020** is present in the system cavity **1008** (FIG. **28**) and at least approximately aligned for receiving the sipper tubes **1050**. The location sensor may detect a flag **1064** of the stage assembly **1046** to determine a level of the stage assembly **1046**. If the flag **1064** has not reached a threshold level along the Z-axis, the location sensor may communicate with the workstation **160** (or other assay system) to notify the user that the tray **1020** is not ready for removal. The workstation **160** could also prevent the user from opening the system door **1010**.

Furthermore, when the distal portions **1052** of the sipper tubes **1050** are initially inserted into the component wells **1060**, the sipper tubes **1050** may pierce protective foils that cover the component wells **1060**. In some instances, the foils may grip the sipper tubes **1050**. When the sipper tubes **1050**

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are subsequently withdrawn from the corresponding component wells **1060**, the gripping of the protective foils may collectively lift the tray **1020**. However, in the illustrated embodiment, the ridges **1035** are configured to grip a tray base **1070** (FIG. **31**) and prevent the tray base **1070** from being lifted in a direction along the Z-axis. For example, the ridges **1035** may grip a lip **1071** of the tray base **1070**.

FIGS. **31-34** illustrate different views of the tray **1020**. The tray **1020** is configured to hold a plurality of component wells **1060**. The component wells **1060** may include various reaction components, such as, but not limited to, one or more samples, polymerases, primers, denaturants, linearization mixes for linearizing DNA, enzymes suitable for a particular assay (e.g., cluster amplification or SBS), nucleotides, cleavage mixes, oxidizing protectants, and other reagents. In some embodiments, the tray **1020** may hold all fluids that are necessary to perform a predetermined assay. In particular embodiments, the tray **1020** may hold all reaction components necessary for generating a sample (e.g., DNA clusters) within a flow cell and performing sample analysis (e.g., SBS). The assay may be performed without removing or replacing any of the component wells **1060**.

The component wells **1060** include rectangular component wells **1060A** (shown in FIGS. **35-36**) and tubular component wells **1060B** (shown in FIG. **37**). The tray **1020** includes a tray base **1070** and a tray cover **1072** coupled to the tray base **1070**. As shown in FIGS. **31** and **32**, the tray cover **1072** includes a handle **1074** that is sized and shaped to be gripped by a user of the tray **1020**. The tray cover **1072** may also include a grip recess **1076** that is sized and shaped to receive one or more fingers of the user.

As shown in FIGS. **31** and **32**, the tray cover **1072** may include a plurality of tube openings **1080** that are aligned with corresponding component wells **1060**. The tube openings **1080** may be shaped to direct the sipper tubes **1050** (exemplary sipper tubes **1050** are shown in FIG. **31**) into the corresponding component wells **1060**. As shown in FIG. **32**, the tray cover **1072** also includes a pin opening **1082** that is sized and shaped to receive the guide pin **1058**. The guide pin **1058** is configured to provide minor adjustments to the position of the tray **1020** if the guide pin **1058** approaches and enters the pin opening **1082** in a misaligned manner. Also shown, the tray **1020** may include an identification tag **1084** along a surface of the tray cover **1072**. The identification tag **1084** is configured to be detected by a reader to provide the user with information regarding the fluids held by the component wells **1060**.

As shown in FIGS. **33** and **34**, the tube openings **1080** are at least partially defined by rims **1086** that project from a surface **1073** of the tray cover **1072**. The rims **1086** project a small distance away from the surface **1073** to prevent inadvertent mixing of fluids that are accidentally deposited onto the tray cover **1072**. Likewise, the identification tag **1084** may be attached to a raised portion **1088** of the tray cover **1072**. The raised portion **1088** may also protect the identification tag **1084** from inadvertently contacting fluids.

FIG. **35** shows a side cross-sectional view of the component well **1060A**, and FIG. **36** shows a bottom perspective view of the component well **1060A**. As shown, the component well **1060A** includes opposite first and second ends **1091** and **1092** and a reservoir **1090** (FIG. **35**) extending therebetween. The reservoir **1090** has a depth  $D_R$  (FIG. **35**) that increases as the reservoir **1090** extends from the second end **1092** to the first end **1091**. The component well **1060A** is configured to receive the sipper tube **1050** in a deeper portion of the reservoir **1090**. As shown in FIG. **36**, the component well **1060A** includes a plurality of projections



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1094 along an exterior surface that are configured to rest upon a surface of the tray base 1070.

FIG. 37 is a perspective view of the component well 1060B. As shown, the component well 1060B may also include a plurality of projections 1096 around an exterior surface of the component well 1060B. The component well 1060B extends along a longitudinal axis 1097 and has a profile that tapers as the component well 1060B extends longitudinally to a bottom 1098. The bottom 1098 may have a substantially planar surface.

FIG. 61 illustrates a method 960 for performing an assay for biological or chemical analysis. In some embodiments, the assay may include a sample generation protocol and a sample analysis protocol. For example, the sample generation protocol may include generating clusters of DNA through bridge amplification and the sample analysis protocol may include sequencing-by-synthesis (SBS) analysis using the clusters of DNA. The sample generation and sample analysis operations may be conducted within a common assay system, such as the assay system 100 or the workstation 160, and without user intervention between the operations. For instance, a user may be able to load a fluidic device into the assay system. The assay system may automatically generate a sample for analysis and carry out the steps for performing the analysis.

With respect to FIG. 61, the method 960 includes establishing at 962 a fluid connection between a fluidic device having a sample area and a reaction component storage unit having a plurality of different reaction components. The reaction components may be configured for conducting one or more assays. The fluidic device may be, for example, the fluidic device 300 or the flow cell 200 described above. In some embodiments, the sample area includes a plurality of reaction components (e.g., primers) immobilized thereon. The storage unit may be, for example, the storage unit 1020 described above. The reaction components may include sample-generation components that are configured to be used to generate the sample, and sample-analysis components that are configured to be used to analyze the sample. In particular embodiments, the sample-generation components include reaction components for performing bridge amplification as described above. Furthermore, in particular embodiments, the sample-analysis components include reaction components for performing SBS analysis as described above.

The method 960 also includes generating at 964 a sample at the sample area of the fluidic device. The generating operation 964 may include flowing different sample-generation components to the sample area and controlling reaction conditions at the sample area to generate the sample. For example, a thermocycler may be used to facilitate hybridizing nucleic acids. However, isothermal methods can be used if desired. Furthermore, a flow rate of the fluids may be controlled to permit hybridization or other desired chemical reactions. In particular embodiments, the generating operation 964 includes conducting multiple bridge-amplification cycles to generate a cluster of DNA.

An exemplary protocol for bridge amplification can include the following steps. A flow cell is placed in fluid communication with a reaction component storage unit. The flow cell includes one or more surfaces to which are attached pairs of primers. A solution having a mixture of target nucleic acids of different sequences is contacted with a solid support. The target nucleic acids can have common priming sites that are complementary to the pairs of primers on the flow cell surface such that the target nucleic acids bind to a first primer of the pairs of primers on the flow cell surface.

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An extension solution containing polymerase and nucleotides can be introduced to the flow cell such that a first amplification product, which is complementary to the target nucleic acid, is formed by extension of the first primer. The extension solution can be removed and replaced with a denaturation solution. The denaturation solution can include chemical denaturants such as sodium hydroxide and/or formamide. The resulting denaturation conditions release the original strand of the target nucleic acid, which can then be removed from the flow cell by removing the denaturation solution and replacing it with the extension solution. In the presence of the extension solution the first amplification product, which is attached to the support, can then hybridize with a second primer of the primer pairs attached to the flow cell surface and a second amplification product comprising an attached nucleic acid sequence complementary to the first amplification product can be formed by extension of the second primer. Repeated delivery of the denaturation solution and extension solution can be used to form clusters of the target nucleic acid at discrete locations on the surface of the flow cell. Although the above protocol is exemplified using chemical denaturation, it will be understood that thermal denaturation can be carried out instead albeit with similar primers and target nucleic acids. Further description of amplification methods that can be used to produce clusters of immobilized nucleic acid molecules is provided, for example, in U.S. Pat. No. 7,115,400; U.S. Publication No. 2005/0100900; WO 00/18957; or WO 98/44151, each of which is incorporated by reference herein.

The method 960 also includes analyzing at 966 the sample at the sample area. Generally, the analyzing operation 966 may include detecting any detectable characteristic at the sample area. In particular embodiments, the analyzing operation 966 includes flowing at least one sample-analysis component to the sample area. The sample-analysis component may react with the sample to provide optically detectable signals that are indicative of an event-of-interest (or desired reaction). For example, the sample-analysis components may be fluorescently-labeled nucleotides used during SBS analysis. When excitation light is incident upon the sample having fluorescently-labeled nucleotides incorporated therein, the nucleotides may emit optical signals that are indicative of the type of nucleotide (A, G, C, or T), and the imaging system may detect the optical signals.

A particularly useful SBS protocol exploits modified nucleotides having removable 3' blocks, for example, as described in WO 04/018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. Repeated cycles of SBS reagents can be delivered to a flow cell having target nucleic acids attached thereto, for example, as a result of the bridge amplification protocol set forth above. The nucleic acid clusters can be converted to single stranded form using a linearization solution. The linearization solution can contain, for example, a restriction endonuclease capable of cleaving one strand of each cluster. Other methods of cleavage can be used as an alternative to restriction enzymes or nicking enzymes, including inter alia chemical cleavage (e.g., cleavage of a diol linkage with periodate), cleavage of abasic sites by cleavage with endonuclease (for example 'USER', as supplied by NEB, Ipswich, Mass., USA, part number M5505S), by exposure to heat or alkali, cleavage of ribonucleotides incorporated into amplification products otherwise comprised of deoxyribonucleotides, photochemical cleavage or cleavage of a peptide linker. After the linearization step a sequencing primer can be delivered to the flow cell under conditions for



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hybridization of the sequencing primer to the target nucleic acids that are to be sequenced.

The flow cell can then be contacted with an SBS extension reagent having modified nucleotides with removable 3' blocks and fluorescent labels under conditions to extend a primer hybridized to each target nucleic acid by a single nucleotide addition. Only a single nucleotide is added to each primer because once the modified nucleotide has been incorporated into the growing polynucleotide chain complementary to the region of the template being sequenced there is no free 3'-OH group available to direct further sequence extension and therefore the polymerase cannot add further nucleotides. The SBS extension reagent can be removed and replaced with scan reagent containing components that protect the sample under excitation with radiation. Exemplary components for scan reagent are described in US publication US 2008/0280773 A1 and US Ser. No. 13/018,255, each of which is incorporated herein by reference. The extended nucleic acids can then be fluorescently detected in the presence of scan reagent. Once the fluorescence has been detected, the 3' block may be removed using a deblock reagent that is appropriate to the blocking group used. Exemplary deblock reagents that are useful for respective blocking groups are described in WO04018497, US 2007/0166705A1 and U.S. Pat. No. 7,057,026, each of which is incorporated herein by reference. The deblock reagent can be washed away leaving target nucleic acids hybridized to extended primers having 3' OH groups that are now competent for addition of a further nucleotide. Accordingly the cycles of adding extension reagent, scan reagent, and deblock reagent, with optional washes between one or more of the steps, can be repeated until a desired sequence is obtained. The above cycles can be carried out using a single extension reagent delivery step per cycle when each of the modified nucleotides has a different label attached thereto, known to correspond to the particular base. The different labels facilitate discrimination between the bases added during each incorporation step. Alternatively, each cycle can include separate steps of extension reagent delivery followed by separate steps of scan reagent delivery and detection, in which case two or more of the nucleotides can have the same label and can be distinguished based on the known order of delivery.

Continuing with the example of nucleic acid clusters in a flow cell, the nucleic acids can be further treated to obtain a second read from the opposite end in a method known as paired end sequencing. Methodology for paired end sequencing are described in PCT publication WO07010252, PCT application Serial No. PCTGB2007/003798 and US patent application publication US 2009/0088327, each of which is incorporated by reference herein. In one example, a series of steps may be performed as follows; generate clusters as set forth above, linearize as set forth above, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above, "invert" the target nucleic acids on the flow cell surface by synthesizing a complementary copy, linearize the resynthesized strand, hybridize a first sequencing primer and carry out repeated cycles of extension, scanning and deblocking, also as set forth above. The inversion step can be carried out by delivering reagents as set forth above for a single cycle of bridge amplification.

Although the analyzing operation has been exemplified above with respect to a particular SBS protocol, it will be understood that other protocols for sequencing any of a variety of other molecular analyses can be carried out as desired. Appropriate modification of the apparatus and

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methods to accommodate various analyses will be apparent in view of the teaching set forth herein and that which is known about the particular analysis method.

In some embodiments, the method 960 is configured to be conducted with minimal user intervention. The generating and analyzing operations 964 and 966 may be conducted in an automated manner by an assay system. For example, in some cases, a user may only load the fluidic device and the storage unit and activate the assay system to perform the method 960. In some embodiments, during the generating and analyzing operations 964 and 966, the storage unit and the fluidic device remain in fluid communication from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. In other words, the fluidic device and the storage unit may remain in fluid communication from before the sample is generated until after the sample is analyzed. In some embodiments, the fluidic device is continuously held by the device holder from a beginning of the generating operation and throughout the analyzing operation until the sample is sufficiently analyzed. During such time, the device holder and an imaging lens may be automatically moved with respect to each other. The storage unit and the fluidic device may remain in fluid communication when the fluidic device and the imaging lens are automatically moved with respect to each other. In some embodiments, the assay system is contained within a workstation housing and the generating and analyzing operations 964 and 966 are conducted exclusively within the workstation housing.

FIG. 38 is a schematic illustration of an optical imaging system 600 formed in accordance with one embodiment. The imaging system 600 includes an optical assembly 602, a light source (or excitation light) module or assembly 604, a flow cell 606 having a sample area 608, and imaging detectors 610 and 612. The light source module 604 includes first and second excitation light sources 614 and 616 that are configured to illuminate the sample area 608 with different excitation spectra. In particular embodiments, the first and second excitation light sources 614 and 616 comprise first and second semiconductor light sources (SLSs). SLSs may include light-emitting diodes (LEDs) or laser diodes. However, other light sources may be used in other embodiments, such as lasers or arc lamps. The first and second SLSs may have fixed positions with respect to the optical assembly 602.

As shown, the optical assembly 602 may include a plurality of optical components. For example, the optical assembly 602 may include lenses 621-627, emission filters 631-634, excitation filters 635 and 636, and mirrors 641-645. The plurality of optical components are arranged to at least one of (a) direct the excitation light toward the sample area 608 of the flow cell 606 or (b) collect emission light from the sample area 608. Also shown, the imaging system 600 may also include a flow system 652 that is in fluid communication with the flow cell 606 and a system controller 654 that is communicatively coupled to the first and second excitation light sources 614 and 616 and the flow system 652. The controller 654 is configured to activate the flow system 652 to flow reagents to the sample area 608 and activate the first and second SLSs after a predetermined time period.

For example, FIG. 60 illustrates a method 900 for performing an assay for biological or chemical analysis. In particular embodiments, the assay may include a sequencing-by-synthesis (SBS) protocol. The method 900 includes flowing reagents through a flow channel of a flow cell at 902. The flow cell may have a sample area that includes a sample



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with biomolecules configured to chemically react with the reagents. The method 900 also includes illuminating the sample area at 904 with first and second semiconductor light sources (SLSs). The first and second SLSs provide first and second excitation spectra, respectively. The biomolecules of the sample may provide light emissions that are indicative of a binding reaction when illuminated by the first or second SLSs. Furthermore, the method 900 includes detecting the light emissions from the sample area at 906. Optionally, the method 900 may include moving the flow cell at 908 relative to an imaging lens and repeating the illuminating and detecting operations 904 and 906. The steps shown in FIG. 60 and exemplified above can be repeated for multiple cycles of a sequencing method.

FIGS. 39 and 40 illustrate various features of a motion-control system 700 formed in accordance with one embodiment that may be used with the imaging system 600. The motion-control system 700 includes an optical base plate 702 and a sample deck 708 that is movably coupled to the base plate 702. As shown, the base plate 702 has a support side 704 and a bottom side 705. The support and bottom sides 704 and 705 face in opposite directions along the Z-axis. The base plate 702 is configured to support a majority of the optical components of the optical assembly 602 (FIG. 38) on the support side 704. The base plate 702 and the sample deck 708 may be movably coupled to each other by an intermediate support 715 and a face plate 722 such that the sample holder 650 may substantially rotate about the X and Y axes, shift along the Y axis, and slide along the X axis.

FIG. 40 is an isolated perspective view of the intermediate support 715, a motor assembly 724, and a movable platform 726 of the sample deck 708 (FIG. 39). The motor assembly 724 is operatively coupled to the platform 726 and is configured to slide the platform 726 bi-directionally along the X-axis. As shown, the intermediate support 715 includes a tail end 728 and an imaging end 730. The intermediate support 715 may include pins 746 and 748 proximate to the imaging end 730 that project away from each other along the Y-axis. Proximate to the imaging end 730, the intermediate support 715 may include a lens opening 750 that is sized and shaped to allow the imaging lens 623 (FIG. 38) to extend therethrough. In the illustrated embodiment, the pins 746 and 748 have a common line 755 extending therethrough that also extends through the lens opening 750.

Returning to FIG. 39, the platform 726 is coupled to the bottom side 705 through the intermediate support 715. Accordingly, a weight of the sample deck 708 may be supported by the base plate 702. Furthermore, the motion-control system 700 may include a plurality of alignment devices 733, 735, 737, and 739 that are configured to position the sample holder 650. In the illustrated embodiment, the alignment devices 733, 735, 737, and 739 are micrometers. The alignment device 733 is operatively coupled to the tail end 728 of the intermediate support 715. When the alignment device 733 is activated, the tail end 728 may be moved in a direction along the Z-axis. Consequently, the intermediate support 715 may rotate about the pins 746 and 748 (FIG. 40) or, more specifically, about the line 755. When the alignment devices 735 and 737 are activated, the sample holder 650 may shift along the Y-axis as directed. When the alignment device 739 is activated, the sample holder 650 may rotate about an axis of rotation R7 that extends parallel to the X-axis.

FIGS. 41-42 show a perspective view and plan view, respectively, of the optical base plate 702 that may be used with the imaging system 600 (FIG. 38). In some embodi-

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ments of the imaging system 600, one or more of the optical components 621-627, 631-636, and 641-645 (FIG. 38) can have a fixed position in the optical assembly 602 such that the fixed (or static) optical component does not move during operation of the imaging system 600. For example, the base plate 702 is configured to support a plurality of optical components and other parts of the imaging system 600. As shown, the base plate 702 constitutes a substantially unitary structure having a support side (or surface) 704 that faces in a direction along the Z-axis. In the illustrated embodiment, the support side 704 is not continuously smooth, but may have various platforms 716-718, depressions (or receiving spaces) 719-721, and component-receiving spaces 711-714 that are located to arrange the optical assembly 602 in a predetermined configuration. As shown in FIG. 42, each of the component-receiving spaces 711-714 has respective reference surfaces 781-784. In some embodiments, the reference surfaces 781-784 can facilitate orienting and holding corresponding optical components in desired positions.

FIGS. 43 and 44 show a front perspective view and a cutaway rear perspective view, respectively, of an optical device 732. As shown in FIG. 43, the optical device 732 is oriented relative to mutually perpendicular axes 791-793. The axis 791 may extend along a gravitational force direction and/or parallel to the Z-axis illustrated above. In particular embodiments, the optical device 732 is configured to be positioned within the component-receiving space 713 (FIG. 43) of the base plate 702 (only a portion of the base plate 702 is shown in FIGS. 43 and 44).

The component-receiving space 713 has one or more surfaces that define an accessible spatial region where an optical component may be held. These one or more surfaces may include the reference surface(s) described below. In the illustrated embodiment, the component-receiving space 713 is a component cavity of the base plate 701 that extends a depth within the base plate 702. However, the base plate 702 may form the component-receiving space in other manners. For example, in a similar way that the base plate 702 may form a cavity, the base plate 702 may also have one or more raised platforms including surfaces that surround and define the component-receiving space. Accordingly, the base plate 702 may be shaped to partially or exclusively provide the component-receiving space. The base plate 702 may include the reference surface. In alternative embodiments, sidewalls may be mounted on the base plate 702 and configured to define the spatial region. Furthermore, other optical devices mounted to the base plate 702 may define the component-receiving spaces. As used herein, when an element "defines" a component-receiving space, the element may exclusively define the component-receiving space or may only partially define the component-receiving space.

The optical device 732 can be removably mounted to the base plate 702 in the component-receiving space 713, but may be configured to remain in a fixed position during operation of the imaging system. However, in alternative embodiment, the optical device 732 is not positioned within the component-receiving space 713, but may be positioned elsewhere, such as on a platform of the support side 704. In the illustrated embodiment, the optical device 732 includes a mounting device 734 and an optical component 736 that is configured to reflect and/or transmit light therethrough. The mounting device 734 is configured to facilitate holding the optical component 736 in a desired orientation and also removably mount the optical component 736 to the base plate 702. The mounting device 734 includes a component retainer 738 and a biasing element 740 that is operatively coupled to the retainer 738.



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In the illustrated embodiment, the optical component **736** comprises an optical filter that transmits optical signals therethrough while filtering for a predetermined spectrum. However, other optical components may be used in alternative embodiments, such as lenses or mirrors. As shown, the optical component **736** may include optical surfaces **742** and **744** that face in opposite directions and define a thickness  $T_3$  of the optical component **736** therebetween. As shown, the optical surfaces **742** and **744** may be continuously smooth and planar surfaces that extend parallel to each other such that the thickness  $T_3$  is substantially uniform. However, the optical surfaces **742** and **744** may have other contours in alternative embodiments. The optical component **736** may have a plurality of component edges **751-754** (FIG. **43**) that define a perimeter or periphery. The periphery surrounds the optical surfaces **742** and **744**. As shown, the periphery is substantially rectangular, but other geometries may be used in alternative embodiments (e.g., circular).

The retainer **738** facilitates holding the optical component **736** in a desired orientation. In the illustrated embodiment, the retainer **738** is configured to engage the optical surface **742** and extend around at least a portion of the periphery to retain the optical component **736**. For example, the retainer **738** may include a wall portion **756** and a frame extension **758** that extends from the wall portion **756** along the periphery of the optical component **736** (e.g., the component edge **752** (FIG. **43**)). In the illustrated embodiment, the frame extension **758** may form a bracket that limits movement of the optical component **736**. More specifically, the frame extension **758** may include a proximal arm **760** and a distal arm **762**. The proximal arm **760** extends from the wall portion **756** along the component edge **752** and the axis **791**. The distal arm **762** extends from the proximal arm **760** along the component edge **751**. The distal arm **762** includes a projection or feature **764** that extends toward and engages the optical component **736**. Also shown, the retainer **738** may include a grip member **766** that is located opposite the frame extension **758**. The grip member **766** and the frame extension **758** may cooperate in limiting movement of the optical component **736** along the axis **793**. The retainer **738** may grip a portion of the periphery of the optical component **736**.

As shown in FIGS. **43** and **44**, the wall portion **756** is configured to engage the optical surface **742**. For example, the wall portion **756** has a mating surface **770** (FIG. **43**) that faces the optical component **736**. In some embodiments, the wall portion **756** includes a plurality of orientation features **771-773** (FIG. **43**) along the mating surface **770**. The orientation features **771-773** are configured to directly engage the optical surface **742** of the optical component **736**. When the orientation features **771-773** directly engage the optical surface **742**, the optical surface **742** (and consequently the optical component **736**) is positioned in a desired orientation with respect to the retainer **738**. As shown in FIG. **43**, the reference surface **783** of the component-receiving space **713** also includes a plurality of orientation features **761-763**. The orientation features **761-763** are configured to directly engage the optical surface **744**. Furthermore, the orientation features **761-763** may be arranged such that each of the orientation features **761-763** generally opposes a corresponding one of the orientation features **771-773**.

Also shown in FIG. **44**, the wall portion **756** has a non-mating surface **774** that faces in an opposite direction with respect to the mating surface **770** (FIG. **43**). The wall portion **756** includes an element projection **776** that extends away from the non-mating surface **774** and the optical

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component **736**. The biasing element **740** is configured to couple to the element projection **776**. In the illustrated embodiment, the element projection **776** and the biasing element **740** extend into a slot **778** of the component-receiving space **713**. The slot **778** is sized and shaped to receive the biasing element **740**. The slot **778** has an element surface **780** that engages the biasing element **740**.

FIG. **45** shows an isolated front view of the optical device **732**, and FIG. **46** shows how the optical device **732** may be removably mounted to the base plate **702**. To removably mount the optical component **736**, the optical component **736** may be positioned within a component-receiving space **789** of the mounting device **734** that is generally defined by the wall portion **756** (FIG. **46**), the frame extension **758**, and the grip member **766**. In particular embodiments, when the optical component **736** is positioned within the mounting device **734**, the optical component **736** is freely held within the component-receiving space **789**. For instance, the optical component **736** may not form an interference fit with the retainer **738**. Instead, during a mounting operation, the optical component **736** may be held within the component-receiving space **789** by the wall portion **756**, the frame extension **758**, the grip member **766** and, for example, an individual's hand. However, in alternative embodiments, the optical component **736** may form an interference fit with the retainer **738** or may be confined within a space that is defined only by the retainer **738**.

With respect to FIG. **46**, during the mounting operation, the biasing element **740** may be initially compressed so that the mounting device **734** may clear and be inserted into the component-receiving space **713**. For example, the biasing element **740** may be compressed by an individual's finger to reduce the size of the optical device **732**, or the biasing element **740** may be compressed by first pressing the biasing element **740** against the element surface **780** and then advancing the retainer **738** into the component-receiving space **713**. Once the optical device **732** is placed within the component-receiving space **713**, the stored mechanical energy of the compressed biasing element **740** may move the retainer **738** and the optical component **736** toward the reference surface **783** until the optical surface **744** directly engages the reference surface **783**. More specifically, the optical surface **744** may directly engage the orientation features **761-763** (FIG. **43**) of the reference surface **783**. As shown in FIG. **46**, when the optical component **736** is mounted, a small gap  $G_1$  may exist between the optical surface **742** and the mating surface **770** (FIG. **43**) because of the orientation features **771-773** (FIG. **43**), and a small gap  $G_2$  may exist between the optical surface **744** and the reference surface **783** because of the orientation features **761-763** (FIG. **43**).

In the mounted position, the biasing element **740** provides an alignment force  $F_A$  that holds the optical surface **744** against the reference surface **783**. The optical and reference surfaces **744** and **783** may be configured to position the optical component **736** in a predetermined orientation. The alignment force  $F_A$  is sufficient to hold the optical component **736** in the predetermined orientation throughout operation of the imaging system. In other words, the mounting device **734** and the reference surface **783** may prevent the optical component **736** from moving in a direction along the axis **792**. Furthermore, in the mounted position, the projection **764** (FIG. **43**) may press against the component edge **751** (FIG. **43**) to prevent the optical component **736** from moving in a direction along the axis **791**. The frame extension **758** and the grip member **766** may prevent or limit movement of the optical component **736** in a direction along



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the axis **793**. Accordingly, the component-receiving space **713** and the mounting device **734** may be configured with respect to each other to hold the optical component **736** in a predetermined orientation during imaging sessions.

As shown in FIG. **45**, when the optical component **736** is in the mounted position, a space portion **798** of the optical surface **744** may face and interface with the reference surface **783**, and a path portion **799** of the optical surface **744** may extend beyond the support side **704** into an optical path taken by optical signals. Also shown in FIG. **46**, the component-receiving space **713** may extend a depth  $D_c$  into the base plate **702** from the support side **704**.

The biasing element **740** may comprise any elastic member capable of storing mechanical energy to provide the alignment force  $F_A$ . In the illustrated embodiment, the elastic member comprises a coil spring that pushes the optical surface **744** against the reference surface **783** when compressed. However, in alternative embodiments, the elastic member and the component-receiving space may be configured such that the elastic member pulls the optical surface against the reference surface when extended. For example, a coil spring may have opposite ends in which one end is attached to the element surface in a slot that extends from the reference surface and another end is attached to the retainer. When the coil spring is extended, the coil spring may provide an alignment force that pulls the optical component against the reference surface. In this alternative embodiment, a rubber band may also be used.

In alternative embodiments, the mounting device **734** may be used to affix the optical component **736** to the base plate **702** using an adhesive. More specifically, the optical component **736** may be held against the reference surface **783** by the mounting device **734**. An adhesive may be deposited into the gap  $G_2$  between the optical surface **744** and the reference surface **783**. After the adhesive cures, the mounting device **734** may be removed while the optical component **736** remains affixed to the reference surface **783** by the adhesive.

FIG. **47** is a block diagram illustrating a method **800** of assembling an optical train. The method **800** includes providing an optical base plate at **802** that has a component-receiving space. The base plate and the component-receiving space may be similar to the base plate **702** and the component-receiving space **713** described above. The method **800** also includes inserting an optical component at **804** into the component-receiving space. The optical component may be similar to the optical component **736** described above and include an optical surface that is configured to reflect or transmit light therethrough. The optical surface may have a space portion that faces a reference surface of the component-receiving space and a path portion that extends beyond the support side into an optical path. The method **800** also includes providing an alignment force at **806** that holds the optical surface against the reference surface to orient the optical component. The optical and reference surfaces may be configured to hold the optical component in a predetermined orientation when the alignment force is provided. In some embodiments, the method **800** may also include removing the optical component at **808** and, optionally, inserting a different optical component at **810** into the component-receiving space. The different optical component may have the same or different optical qualities. In other words, the different optical component may be a replacement that has the same optical qualities or the different optical component may have different optical qualities.

FIGS. **48** and **49** provide a perspective view and a side view, respectively, of the light source (or excitation light

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module) **604**. As used herein, a light source module includes one or more light sources (e.g., lasers, arc lamps, LEDs, laser diodes) that are secured to a module frame and also includes one or more optical components (e.g., lenses or filters) that are secured to the module frame in a fixed and predetermined position with respect to said one or more light sources. The light source modules may be configured to be removably coupled within an imaging system so that a user may relatively quickly install or replace the light source module. In particular embodiments, the light source module **604** constitutes a SLS module **604** that includes the first and second SLSs **614** and **616**. As shown, the SLS module **604** includes a module frame **660** and a module cover **662**. A plurality of imaging components may be secured to the module frame **660** in fixed positions with respect to each other. For example, the first and second SLSs **614** and **616**, the excitation filter **635**, and the lenses **624** and **625** may be mounted onto the module frame **660**. In addition, the SLS module **604** may include first and second heat sinks **664** (FIGS. **48**) and **666** that are configured to transfer thermal energy from the first and second SLSs **614** and **616**, respectively.

The SLS module **604** and the module frame **660** may be sized and shaped such that an individual could hold the SLS module **604** with the individual's hands and readily manipulate for installing into the imaging system **600**. As such, the SLS module **604** has a weight that an adult individual could support.

The SLS module **604** is configured to be placed within the module-receiving space **719** (FIG. **41**) and removably coupled to the base plate **702** (FIG. **41**). As shown, the module frame **660** has a plurality of sides including a mounting side **670** and an engagement face **671** (FIG. **48**). In the illustrated embodiment, the module frame **660** is substantially rectangular or block-shaped, but the module frame **660** may have other shapes in alternative embodiments. The mounting side **670** is configured to be mounted to the base plate **702** within the module-receiving space **719**. As such, at least a portion of the module-receiving space **719** may be shaped to receive and hold the SLS module **604**. Similar to the component-receiving space **713**, the module-receiving space **719** may be defined by one or more surfaces that provide an accessible spatial region where the SLS module **604** may be held. The surface(s) may be of the base plate **702**. For example, in the illustrated embodiment, the module-receiving space **719** is a depression of the base plate **702**. The mounting side **670** may have a contour that substantially complements the base plate **702** and, more specifically, the module-receiving space **719**. For example, the mounting side **670** may be substantially planar and include a guidance pin **672** (FIG. **49**) projecting therefrom that is configured to be inserted into a corresponding hole (not shown) in the base plate **702**. The guidance pin **672** may be a fastener (e.g., screw) configured to facilitate removably coupling the module frame **660** to the base plate **702**. In particular embodiments, the guidance pin **672** is inserted into the base plate **702** at a non-orthogonal angle. As shown in FIG. **49**, the heat sink **666** may be coupled to the module frame **660** such that an offset **676** exists from the mounting side **670** to the heat sink **666**.

The module frame **660** may include first and second light passages **682** and **684** that intersect each other at a passage intersection **685**. The SLSs **614** and **616** may be secured to the module frame **660** and have fixed positions with respect to each other. The SLSs **614** and **616** are oriented such that optical signals are substantially directed along optical paths through the respective light passages **682** and **684** toward the



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passage intersection **685**. The optical paths may be directed toward the excitation filter **635**. In the illustrated embodiment, the optical paths are perpendicular to one another until reaching the excitation filter **635**. The excitation filter **635** is oriented to reflect at least a portion of the optical signals generated by the SLS **616** and transmit at least a portion of the optical signals generated by the SLS **614**. As shown, the optical signals from each of the SLSs **614** and **616** are directed along a common path and exit the SLS module **604** through a common module window **674**. The module window **674** extends through the engagement face **671**.

FIG. **50** is a plan view of the SLS module **604** mounted onto the base plate **702**. In the illustrated embodiment, the SLS module **604** is configured to rest on the base plate **702** such that the gravitational force  $g$  facilitates holding the SLS module **604** thereon. As such, the SLS module **604** may provide an integrated device that is readily removed or separated from the optical assembly **600**. For example, after removing a housing (not shown) of the assay system or after receiving access to the optical assembly, the SLS module **604** may be grabbed by an individual and removed or replaced. When the SLS module **604** is located on the base plate **702**, the engagement face **671** may engage an optical device **680**. The optical device **680** may be adjacent to the module window **674** such that the optical signals generated by the SLS module **604** are transmitted through the optical device **680**.

Although the illustrated embodiment is described as using an SLS module with first and second SLSs, excitation light may be directed onto the sample in other manners. For example, the SLS module **604** may include only one SLS and another optical component (e.g., lens or filter) having fixed positions with respect to each other in a module frame. Likewise, more than two SLSs may be used. In a similar manner, light modules may include only one laser or more than two lasers.

However, embodiments described herein are not limited to only having modular excitation systems, such as the SLS module **604**. For example, the imaging system **600** may use a light source that is not mounted to a module frame. More specifically, a laser could be directly mounted to the base plate or other portion of the imaging system or may be mounted to a frame that, in turn, is mounted within the imaging system.

Returning to FIG. **38**, the imaging system **600** may have an image-focusing system **840** that includes the object or sample holder **650**, an optical train **842**, and the imaging detector **610**. The optical train **842** is configured to direct optical signals from the sample holder **650** (e.g., light emissions from the sample area **608** of the flow cell **606**) to a detector surface **844** of the imaging detector **610**. As shown in FIG. **38**, the optical train **842** includes the optical components **623**, **644**, **634**, **633**, **621**, **631**, and **642**. The optical train **842** may include other optical components. In the illustrated configuration, the optical train **842** has an object or sample plane **846** located proximate to the sample holder **650** and an image plane **848** located proximate to the detector surface **844**. The imaging detector **610** is configured to obtain object or sample images at the detector surface **844**.

In some embodiments, the image-focusing system **840** is configured to move the image plane **848** relative to the detector **610** and capture a test image. More specifically, the image plane **848** may be moved such that the image plane **848** extends in a non-parallel manner with respect to the detector surface **844** and intersects the detector surface **844**. A location of the intersection may be determined by ana-

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lyzing the test image. The location may then be used to determine a degree-of-focus of the imaging system **600**. In particular embodiments, the image-focusing system **840** utilizes a rotatable mirror that is operatively coupled to an actuator for moving the rotatable mirror. However, the image-focusing system **840** may move other optical components that direct the optical signals to the detector surface **844**, or the image-focusing system **840** may move the detector **610**. In either case, the image plane **848** may be relatively moved with respect to the detector surface **844**. For example, the image-focusing system **840** may move a lens.

In particular embodiments, the imaging detector **610** is configured to obtain test images using a rotatable mirror **642** to determine a degree-of-focus of the imaging system **600**. As a result of the determined degree-of-focus, the imaging system **600** may move the sample holder **650** so that the object or sample is located within the sample plane **846**. For example, the sample holder **650** may be configured to move the sample area **608** in a  $z$ -direction a predetermined distance (as indicated by  $\Delta z$ ).

FIG. **51** is a plan view that illustrates several of the components in the image-focusing system **840**. As shown, the image-focusing system **840** includes a rotatable mirror assembly **850** that includes the mirror **642**, a mounting assembly **852** having the mirror **642** mounted thereon, and an actuator or rotation mechanism **854** that is configured to rotate the mounting assembly **852** and the mirror **642** about an axis of rotation  $R_6$ . The mirror **642** is configured to reflect optical signals **863** that are received from the sample area **608** (FIG. **38**) toward the imaging detector **610** and onto the detector surface **844**. In the illustrated embodiment, the mirror **642** reflects the optical signals **863** directly onto the detector surface **844** (i.e., there are no intervening optical components that redirect the optical signals **863**). However, in alternative embodiments, there may be additional optical components that affect the propagation of the optical signals **863**.

In the illustrated embodiment, the image-focusing system **840** also includes positive stops **860** and **862** that are configured to prevent the mirror **642** from rotating beyond predetermined rotational positions. The positive stops **860** and **862** have fixed positions with respect to the axis  $R_6$ . The mounting assembly **852** is configured to pivot about the axis  $R_6$  between the positive stops **860** and **862** depending upon whether sample images or test images are being obtained. Accordingly, the mirror **642** may be rotated between a test position (or orientation) and an imaging position (or orientation). By way of example only, the mirror **642** may be rotated from approximately  $5^\circ$  to approximately  $12^\circ$  about the axis  $R_6$  between the different rotational positions. In particular embodiments, the mirror **642** may be rotated approximately  $8^\circ$  about the axis  $R_6$ .

FIG. **52** is a perspective view of the mirror assembly **850**. As shown, the mounting assembly **852** includes an interior frame **864** and a support bracket **866**. The interior frame **864** is configured to couple to the mirror **642** and also to the support bracket **866**. The interior frame **864** and the support bracket **866** may interact with each other and a plurality of set screws **868** to provide minor adjustments to the orientation of the mirror **642**. As such, the mounting assembly **852** may constitute a gimbal mirror mount assembly. Also shown, the mounting assembly **852** is coupled to the rotation mechanism **854**. In the illustrated embodiment, the rotation mechanism **854** comprises a direct drive motor. However, a variety of alternative rotation mechanisms may be used, such as direct current (DC) motors, solenoid drivers, linear



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actuators, piezoelectric motors, and the like. Also shown in FIG. 52, the positive stop 860 may have a fixed position with respect to the rotation mechanism 854 and the axis  $R_6$ .

As discussed above, the rotation mechanism 854 is configured to rotate or pivot the mirror 642 about the axis  $R_6$ . As shown in FIG. 52, the mirror 642 has a geometric center C that extends along the axis  $R_6$ . The geometric center C of the mirror 642 is offset with respect to the axis  $R_6$ . In some embodiments, the rotation mechanism 854 is configured to move the mirror 642 between the test position and imaging position in less than 500 milliseconds. In particular embodiments, the rotation mechanism 854 is configured to move the mirror 642 between the test position and imaging position in less than 250 milliseconds or less than 160 milliseconds.

FIG. 53 is a schematic diagram of the mirror 642 in the imaging position. As shown, the optical signals 863 from the sample area 608 (FIG. 38) are reflected by the mirror 642 and directed toward the detector surface 844 of the imaging detector 610. Depending upon the configuration of the optical train 842 and the z-position of the sample holder 610, the sample area 608 may be sufficiently in-focus or not sufficiently in-focus (i.e., out-of-focus). FIG. 53 illustrates two image planes 848A and 848B. The image plane 848A substantially coincides with the detector surface 844 and, as such, the corresponding sample image has an acceptable or sufficient degree-of-focus. However, the image plane 848B is spaced apart from the detector surface 844. Accordingly, the sample image obtained when the image plane 848B is spaced apart from the detector surface 844 may not have a sufficient degree-of-focus.

FIGS. 54 and 55 illustrate sample images 870 and 872, respectively. The sample image 870 is the image detected by the imaging detector 610 when the image plane 848A coincides with the detector surface 844. The sample image 872 is the image detected by the imaging detector 610 when the image plane 848B does not coincide with the detector surface 844. (The sample images 870 and 872 include clusters of DNA that provide fluorescent light emissions when excited by predetermined excitation spectra.) As shown in FIGS. 54 and 55, the sample image 870 has an acceptable degree-of-focus in which each of the clusters along the sample image 870 is clearly defined, and the sample image 872 does not have an acceptable degree-of-focus in which each of the clusters is clearly defined.

FIG. 56 is a schematic diagram of the mirror 642 in the focusing position. As shown, the mirror 642 in the focusing position has been rotated about the axis  $R_6$  an angle  $\theta$ . Again, the optical signals 863 from the sample area 608 (FIG. 38) are reflected by the mirror 642 and directed toward the detector surface 844 of the imaging detector 610. However, the optical train 842 in FIG. 56 is arranged so that the image plane 848 has been moved with respect to the detector surface 844. More specifically, the image plane 848 does not extend parallel to the detector surface 844 and, instead, intersects the detector surface 844 at a plane intersection PI. While the mirror 642 is in the focusing position, the imaging system 600 may obtain a test image of the sample area 608. As shown in FIG. 56, the plane intersections PI may occur at different locations on the detector surface 844 depending upon the degree to which the sample area 608 is in-focus during an imaging session.

For example, FIGS. 57 and 58 illustrate test images 874 and 876, respectively. The test image 874 represents the image obtained when the sample area 608 is in-focus, and the test image 876 represents the image obtained when the optical train 842 is out-of-focus. As shown, the test image 874 has a focused region or location  $FL_1$  that is located a

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distance  $XD_1$  away from a reference edge 880, and the test image 876 has a focused region or location  $FL_2$  that is located a distance  $XD_2$  away from a reference edge 880. The focused locations  $FL_1$  and  $FL_2$  may be determined by an image analysis module 656 (FIG. 38).

To identify the focused locations  $FL_1$  and  $FL_2$  in the test images 874 and 876, the image analysis module 656 may determine the location of an optimal degree-of-focus in the corresponding test image. More specifically, the analysis module 656 may determine a focus score for different points along the x-dimension of the test images 874 and 876. The analysis module 656 may calculate the focus score at each point based on one or more image quality parameters. Examples of image quality parameters include image contrast, spot size, image signal to noise ratio, and the mean-square-error between pixels within the image. By way of example, when calculating a focus score, the analysis module 656 may calculate a coefficient of variation in contrast within the image. The coefficient of variation in contrast represents an amount of variation between intensities of the pixels in an image or a select portion of an image. As a further example, when calculating a focus score, the analysis module 656 may calculate the size of a spot derived from the image. The spot can be represented as a Gaussian spot and size can be measured as the full width half maximum (FWHM), in which case smaller spot size is typically correlated with improved focus.

After determining the focused location FL in the test image, the analysis module 656 may then measure or determine the distance XD that the focused location FL is spaced apart or separated from the reference edge 880. The distance XD may then be correlated to a z-position of the sample area 608 with respect to the sample plane 846. For example, the analysis module 656 may determine that the distance  $XD_2$  shown in FIG. 58 corresponds to the sample area 608 be located a distance  $\Delta z$  from the sample plane 846. As such, the sample holder 650 may then be moved the distance  $\Delta z$  to move the sample area 608 within the sample plane 846. Accordingly, the focused locations FL in test images may be indicative of a position of the sample area 608 with respect to the sample plane 846. As used herein, the phrase “being indicative of a position of the object (or sample) with respect to the object (or sample) plane” includes using the factor (e.g., the focused location) to provide a more suitable model or algorithm for determining the distance  $\Delta z$ .

FIG. 59 is a block diagram illustrating a method 890 for controlling focus of an optical imaging system. The method 890 includes providing an optical train at 892 having a rotatable mirror that is configured to direct optical signals onto a detector surface. The detector surface may be similar to the detector surface 844. The optical train may have an object plane, such as the sample plane 846, that is proximate to an object. The optical train may also have an image plane, such as the image plane 848, that is proximate to the detector surface. The rotatable mirror may be rotatable between an imaging position and a focusing position.

The method 890 also includes rotating the mirror at 894 to the focusing position and obtaining a test image of the object at 896 when the mirror is in the focusing position. The test image may have an optimal degree-of-focus at a focused location. The focused location may be indicative of a position of the object with respect to the object plane. Furthermore, the method 890 may also include moving the object at 898 toward the object plane based on the focused location.



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It is to be understood that the above description is intended to be illustrative, and not restrictive. For example, the above-described embodiments (and/or aspects thereof) may be used in combination with each other. In addition, many modifications may be made to embodiments without departing from the of the scope invention in order to adapt a particular situation or material. While the specific components and processes described herein are intended to define the parameters of the various embodiments, they are by no means limiting and are exemplary embodiments. Many other embodiments will be apparent to those of skill in the art upon reviewing the above description. The scope of the invention should, therefore, be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. In the appended claims, the terms “including” and “in which” are used as the plain-English equivalents of the respective terms “comprising” and “wherein.” Moreover, in the following claims, the terms “first,” “second,” and “third,” etc. are used merely as labels, and are not intended to impose numerical requirements on their objects. Further, the limitations of the following claims are not written in means-plus-function format and are not intended to be interpreted based on 35 U.S.C. § 112, sixth paragraph, unless and until such claim limitations expressly use the phrase “means for” followed by a statement of function void of further structure.

What is claimed is:

1. A DNA sequencing instrument comprising:

an optical deck comprising a light source assembly and two imaging detectors;

a sample deck comprising a slidable platform configured to support a fluidic device comprising a flow cell, the sample deck further comprising a thermal module configured to control a temperature of the flow cell;

a fluid storage system comprising:

an enclosure having a cavity;

a door configured to open to provide access to the cavity;

a temperature control assembly configured to regulate a temperature within the cavity, the temperature control assembly comprising a thermoelectric cooling assembly located at a rear of the cavity opposite the door;

a fluid removal assembly comprising:

an elevator mechanism including a drive motor and a lead screw operatively coupled to the drive motor,

a transport platform carrying a flag and configured to hold an array of sipper tubes, the elevator mechanism configured to move the array of sipper tubes bi-directionally between a withdrawn level and a deposited level,

a guide plate having openings through which the array of sipper tubes slide,

a plurality of support beams coupled to the guide plate and extending parallel to the lead screw, and a location sensor configured to determine a level of the array of sipper tubes;

a reaction component tray within the cavity of the enclosure of the fluid storage system, wherein the reaction component tray comprises a plurality of component wells configured to store fluids, wherein the plurality of

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component wells include a polymerase, modified nucleotides, a cleavage mix, and an oxidizing protectant; and

a pump configured to direct the flow of fluids from the reaction component tray through the array of sipper tubes to a multi-port valve, the multi-port valve configured to selectively flow the fluids from the reaction component tray to the sample deck during DNA sequencing on the flow cell,

wherein rotation of the lead screw in a first direction moves the transport platform toward the guide plate until the array of sipper tubes is at the deposited level, wherein rotation of the lead screw in a second, opposite direction moves the transport platform away from the guide plate until the array of sipper tubes is at the withdrawn level, wherein sipper tubes of the array of sipper tubes include distal portions that are inserted into component wells of the reaction component tray when the array of sipper tubes is at the deposited level, wherein the distal portions of the sipper tubes are completely removed from the component wells when the array of sipper tubes is at the withdrawn level, and wherein the location sensor is configured to detect the flag and determine when the array of sipper tubes has not reached a threshold level such that the reaction component tray is not ready for removal from the cavity.

2. The DNA sequencing instrument of claim 1, wherein the pump is configured to direct a flow of the modified nucleotides from the reaction component tray through a sipper tube of the array of sipper tubes to the multi-port valve, the multi-port valve configured to selectively flow the modified nucleotides to the sample deck during DNA sequencing on the flow cell,

wherein the polymerase and the modified nucleotides are stored in a first component well of the reaction component tray, and wherein the multi-port valve is configured to selectively flow the polymerase and the modified nucleotides to the sample deck during DNA sequencing on the flow cell, and

wherein the pump is configured to direct a flow of the cleavage mix from a second component well of the reaction component tray through a sipper tube of the array of sipper tubes to the multi-port valve, the multi-port valve configured to selectively flow the cleavage mix to the sample deck during DNA sequencing on the flow cell.

3. The DNA sequencing instrument of claim 1, wherein the fluidic device comprises a housing that includes a reception space and the flow cell located within the reception space, and

wherein the sample deck comprises a support structure having alignment members, wherein the housing comprises recesses that are located adjacent to the reception space, and wherein the alignment members are inserted through corresponding recesses of the housing.

4. The DNA sequencing instrument of claim 1, further comprising a casing enclosing the optical deck and the fluid storage system therein, the door providing access through the casing to the cavity, wherein the enclosure of the fluid storage system is separate from the optical deck and separate from the sample deck. control a temperature of the flow cell.

\* \* \* \* \*



UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**


PATENT NO. : 11,697,116 B2  
APPLICATION NO. : 17/714129  
DATED : July 11, 2023  
INVENTOR(S) : Erik Williamson et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In Column 54, Line 62, Claim 4, after “deck.” delete “control a temperature of the flow cell.”,  
therefor.

Signed and Sealed this  
Fifth Day of September, 2023  
  
Katherine Kelly Vidal  
*Director of the United States Patent and Trademark Office*



# EXHIBIT 6





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(12) **United States Patent**  
**Kaplan et al.**

(10) **Patent No.: US 12,251,702 B2**  
(45) **Date of Patent: Mar. 18, 2025**

(54) **FLOWCELL CARTRIDGE WITH FLOATING SEAL BRACKET**

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(60) Continuation of application No. 18/167,836, filed on Feb. 11, 2023, now Pat. No. 12,097,502, which is a  
(Continued)

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**B01L 9/00** (2006.01)  
**B01L 3/00** (2006.01)

(52) **U.S. Cl.**  
CPC ..... **B01L 9/527** (2013.01); **B01L 3/502715** (2013.01); **B01L 2200/025** (2013.01);  
(Continued)

(58) **Field of Classification Search**

CPC ..... B01L 3/502715; B01L 9/527; B01L 2200/025; B01L 2200/027; B01L 2200/04;

(Continued)

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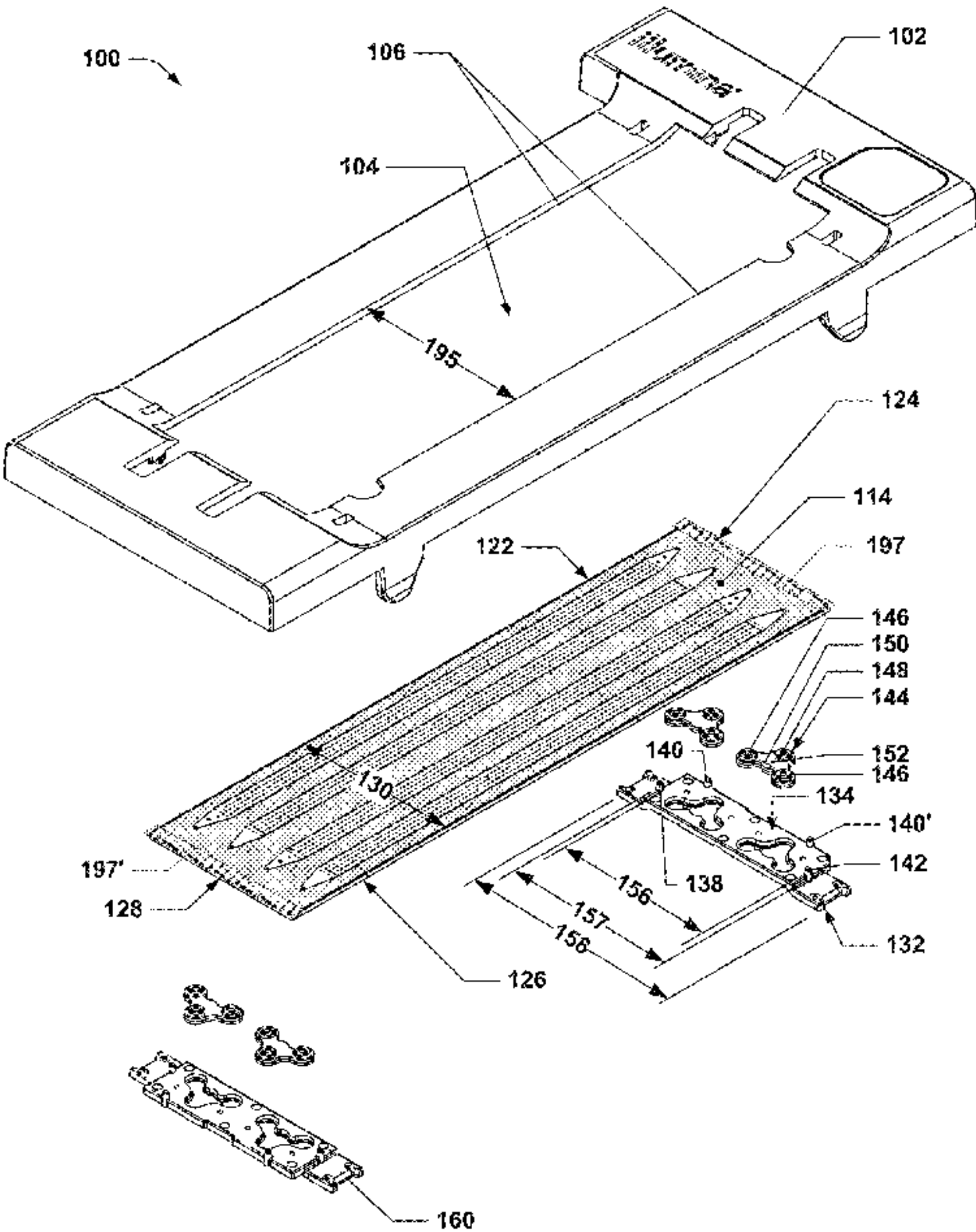
*Primary Examiner* — Dean Kwak

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(57) **ABSTRACT**

A cartridge for use with chemical or biological analysis systems, as well as methods of using the same, is provided. The cartridge may include a floating microfluidic plate that is held in the cartridge using one or more floating support brackets that incorporate gaskets that may seal against fluidic ports on the microfluidic plate. The floating support brackets may include indexing features that may align the microfluidic plate with the seals.

**11 Claims, 9 Drawing Sheets**





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Related U.S. Application Data

division of application No. 16/777,881, filed on Jan. 30, 2020, now Pat. No. 11,577,253, which is a division of application No. 16/436,485, filed on Jun. 10, 2019, now Pat. No. 10,549,282, which is a continuation of application No. 15/841,109, filed on Dec. 13, 2017, now Pat. No. 10,357,775.

(60) Provisional application No. 62/441,927, filed on Jan. 3, 2017.

(52) U.S. Cl.

CPC ..... *B01L 2200/027* (2013.01); *B01L 2200/04* (2013.01); *B01L 2200/0689* (2013.01); *B01L 2300/041* (2013.01); *B01L 2300/0609* (2013.01); *B01L 2300/0809* (2013.01); *B01L 2300/0816* (2013.01); *B01L 2300/0822* (2013.01); *B01L 2300/0877* (2013.01)

(58) Field of Classification Search

CPC ..... B01L 2200/0689; B01L 2300/041; B01L 2300/0877; B01L 2300/022; B01L 2300/043; B01L 2300/0609; B01L 2300/0809; B01L 2300/0816; B01L 7/52; B01L 2300/0822

See application file for complete search history.

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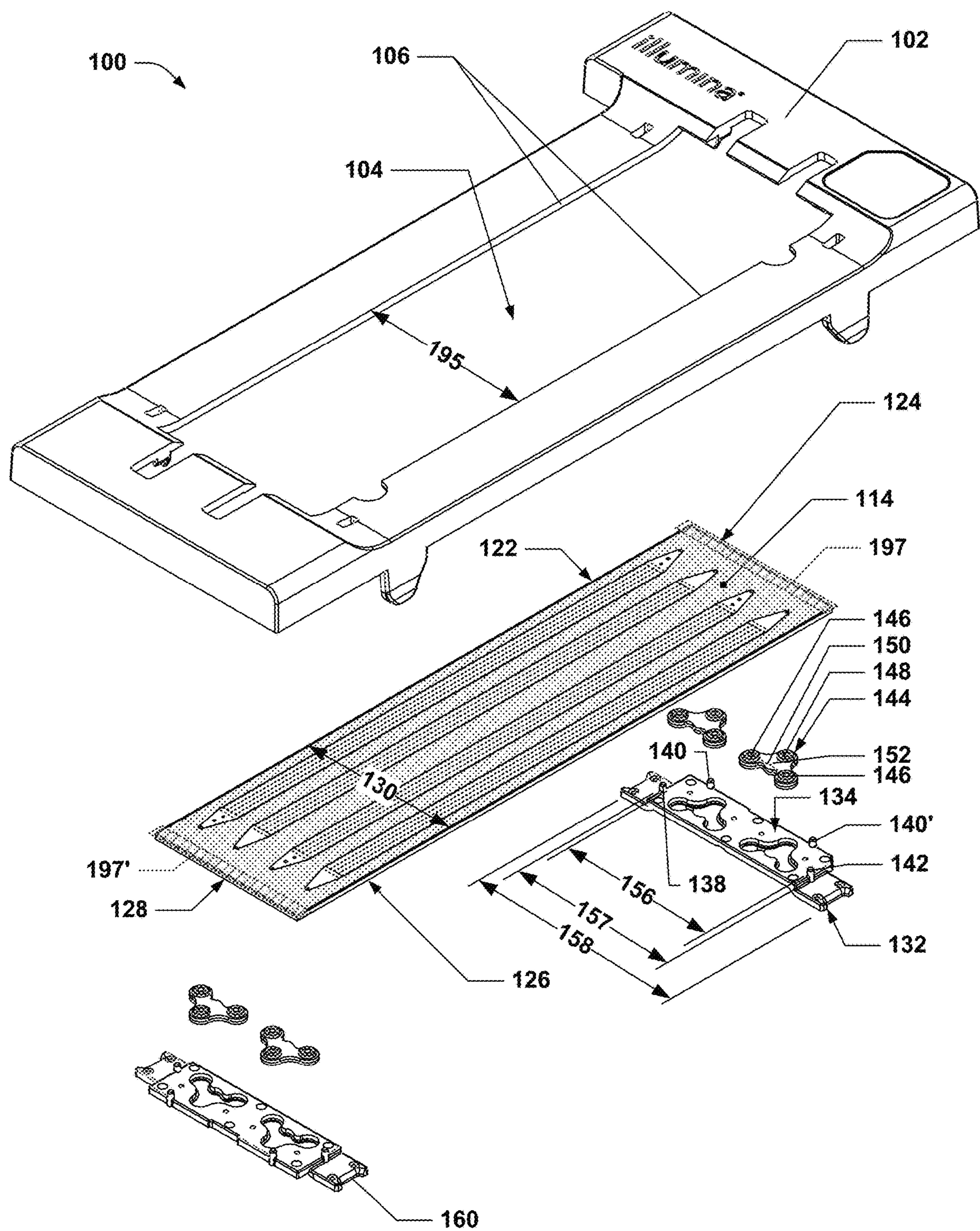
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## Figure 1



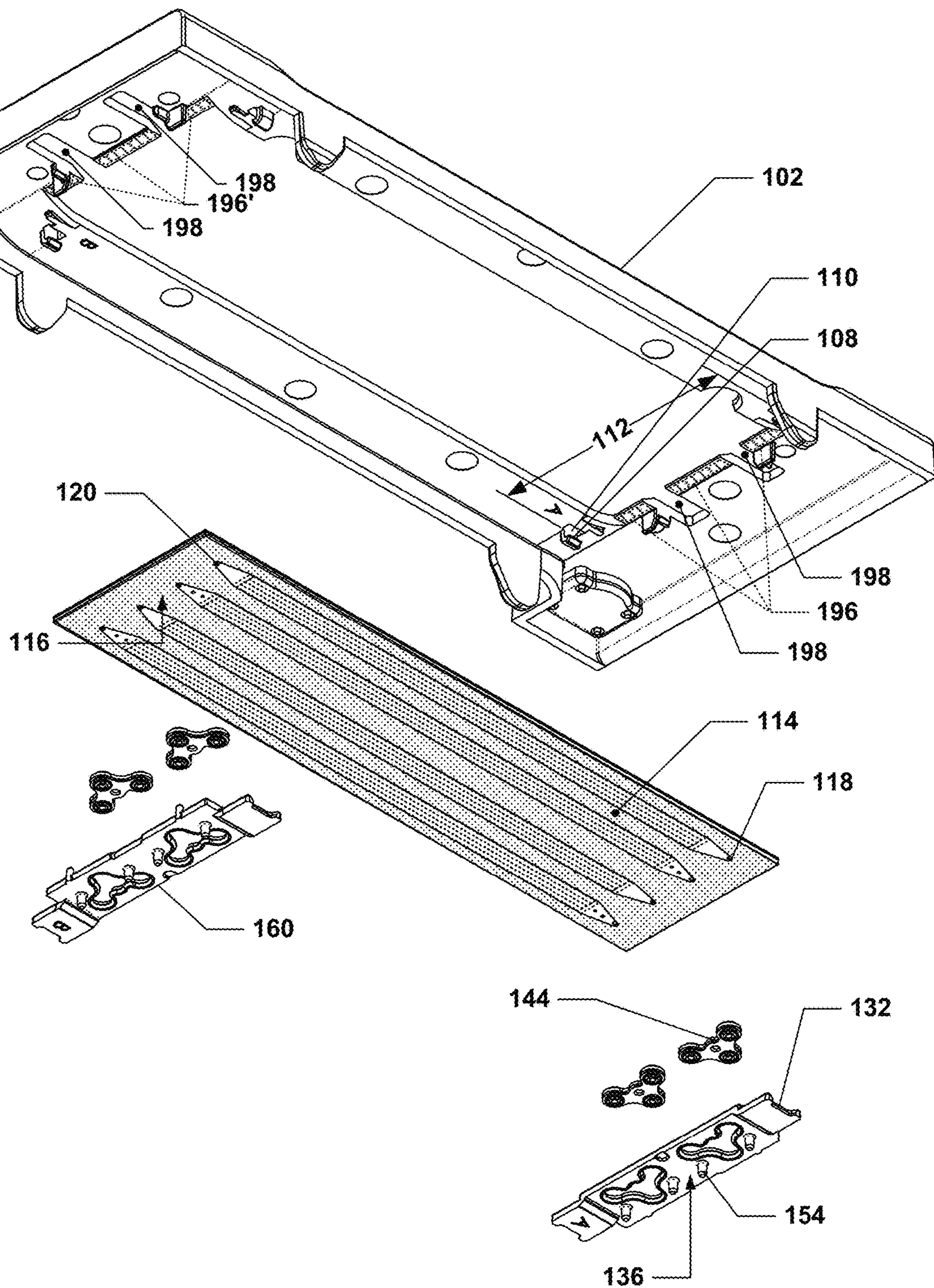


Figure 2







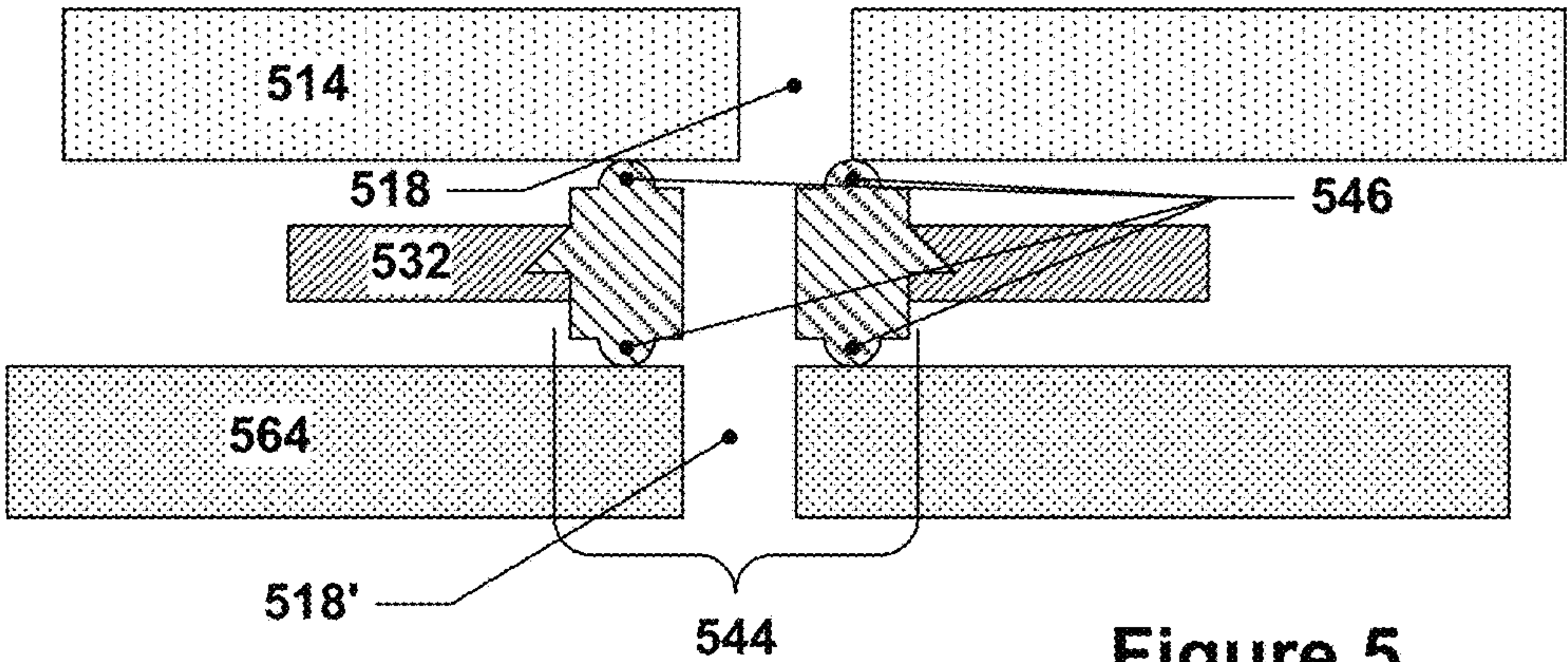


Figure 5

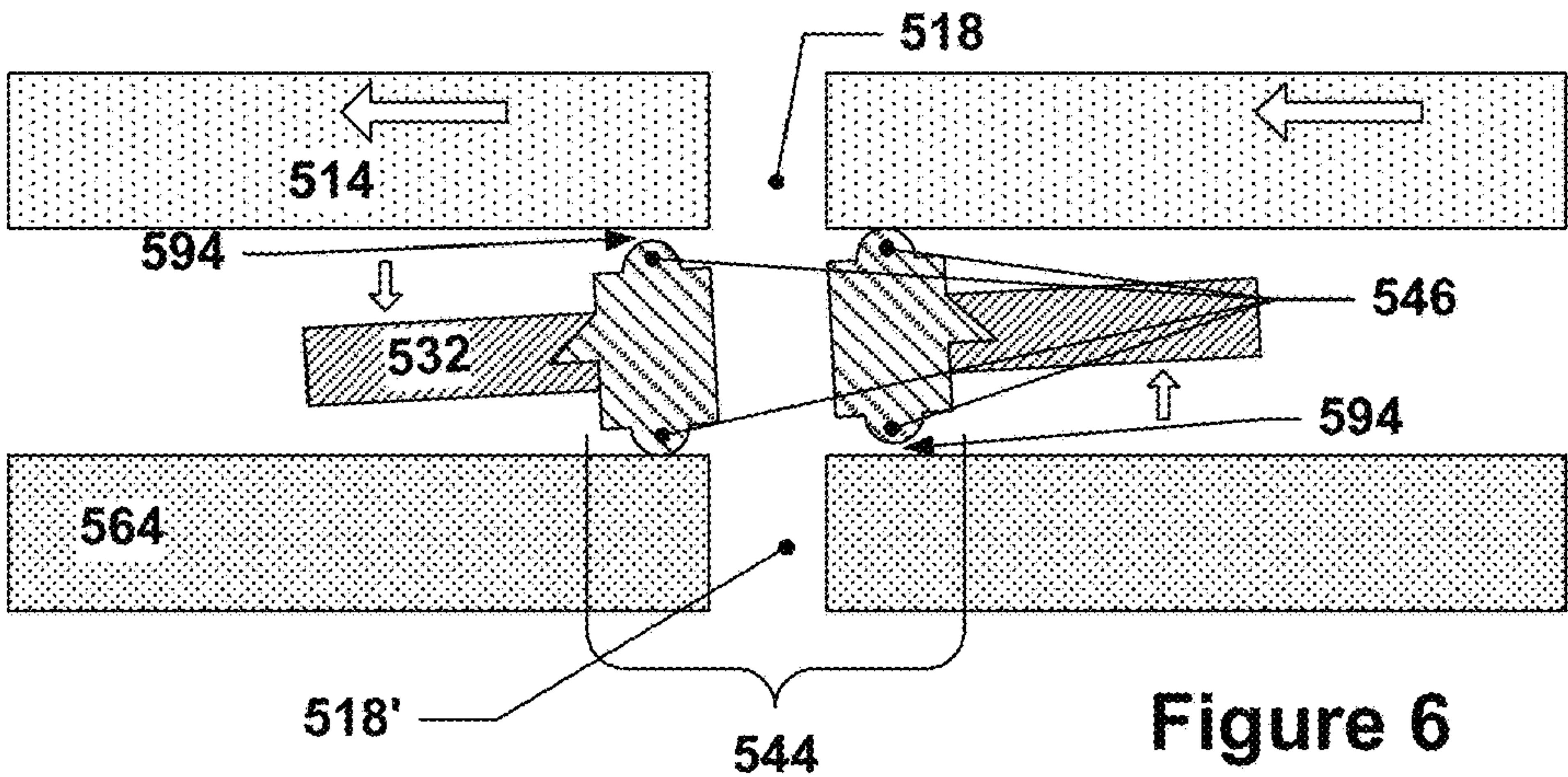


Figure 6

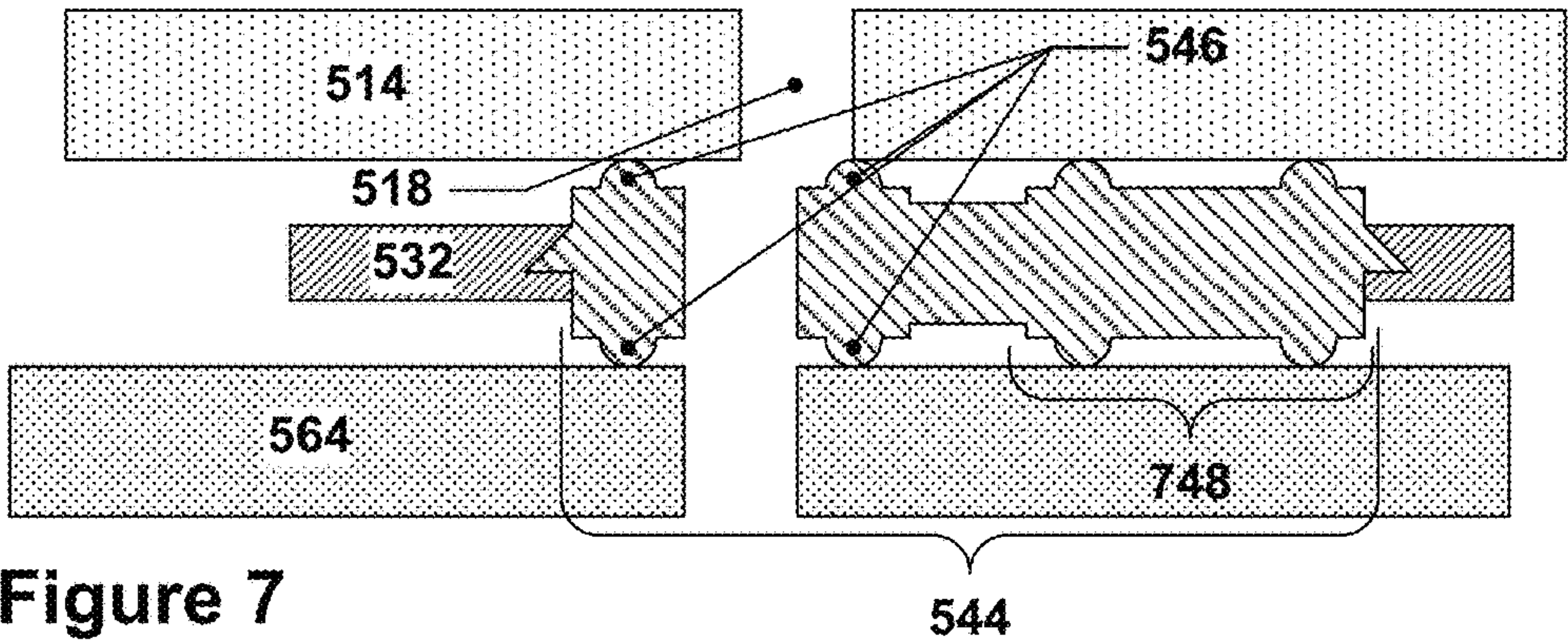


Figure 7

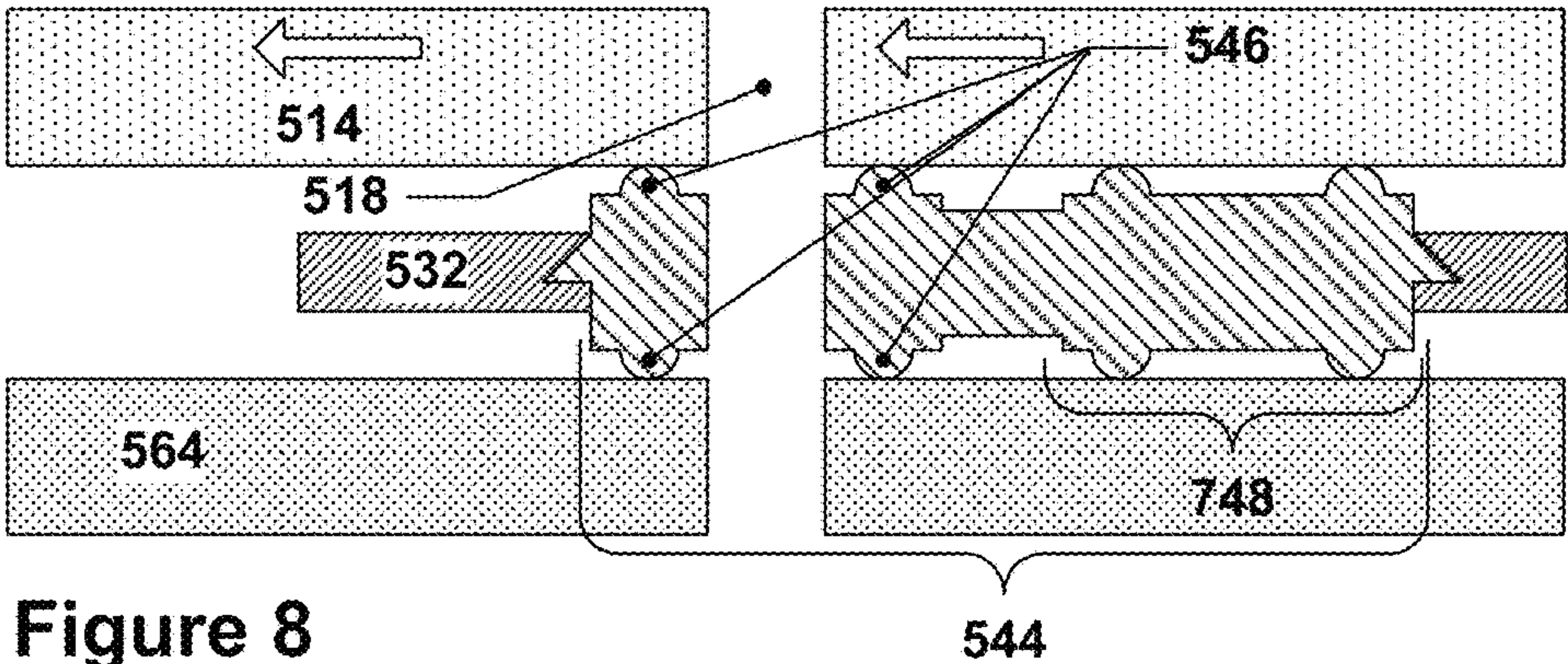
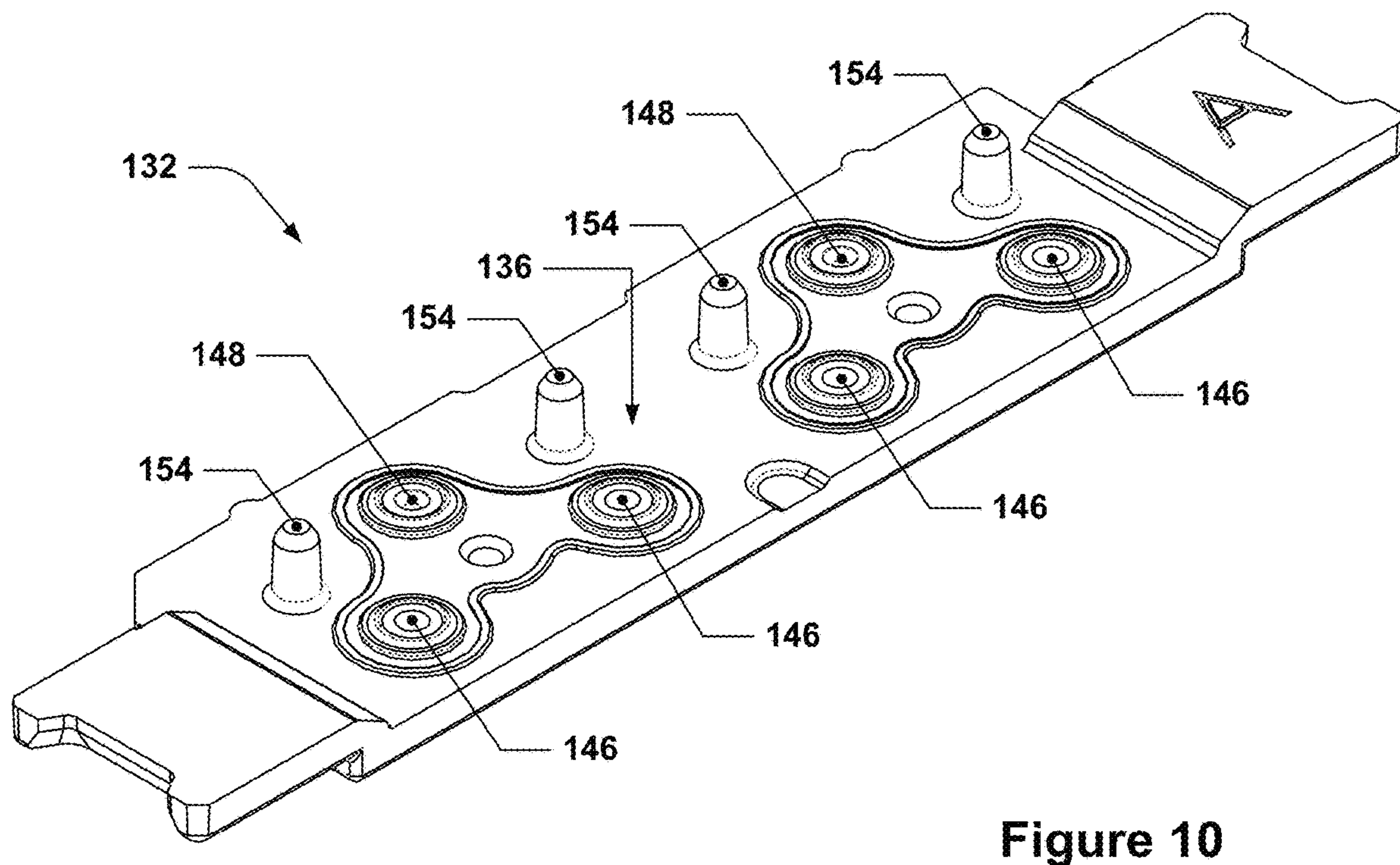
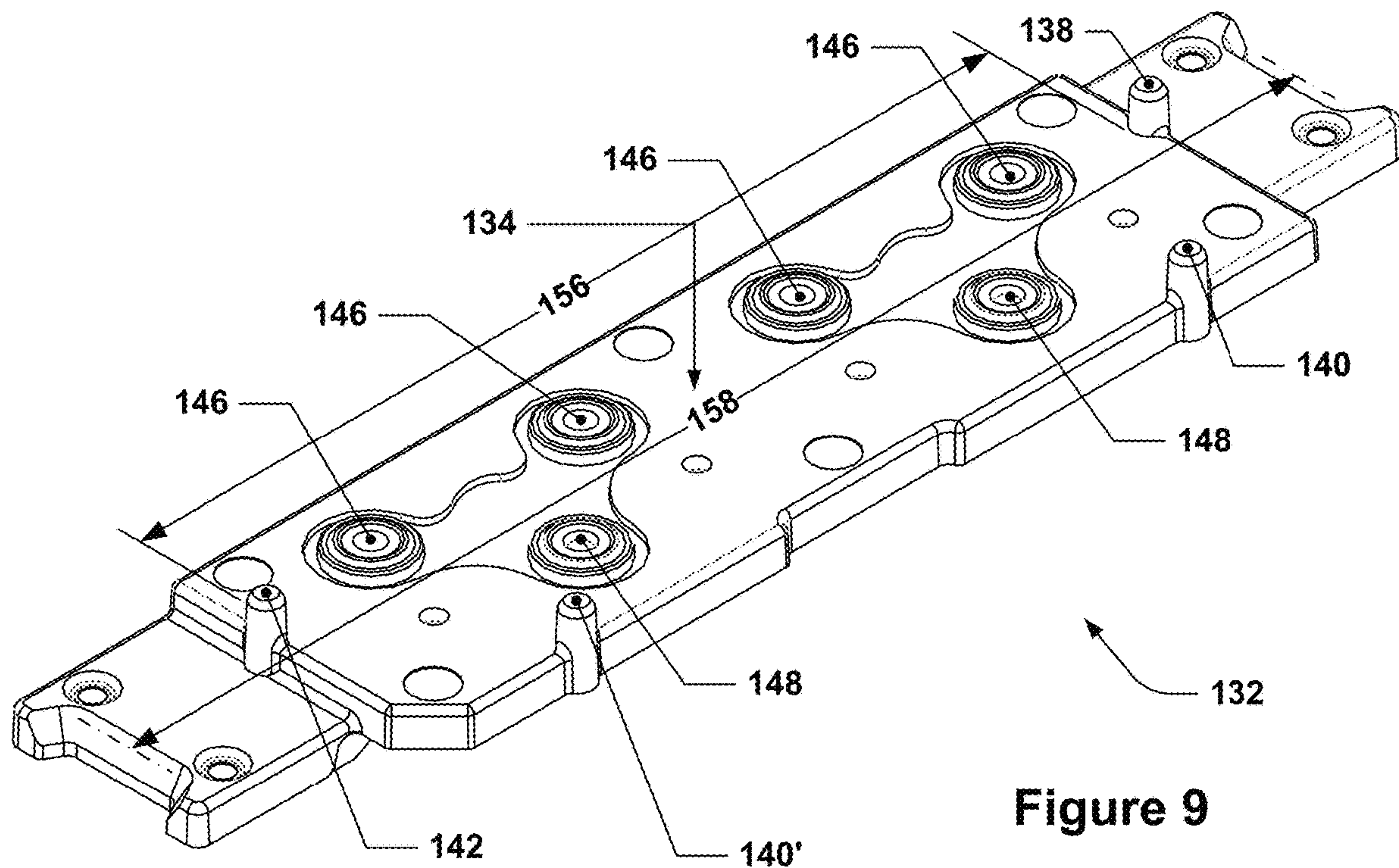


Figure 8







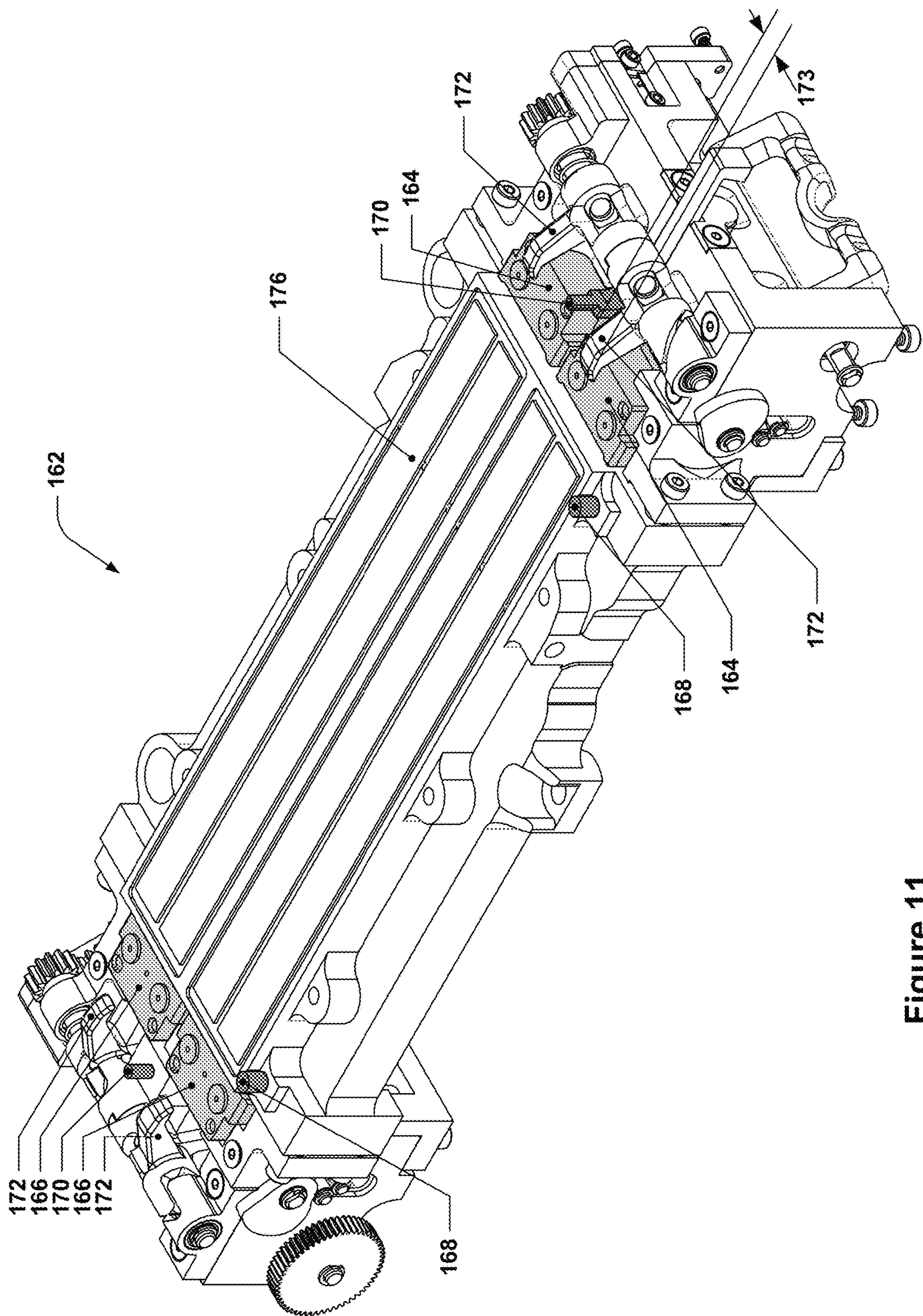


Figure 11



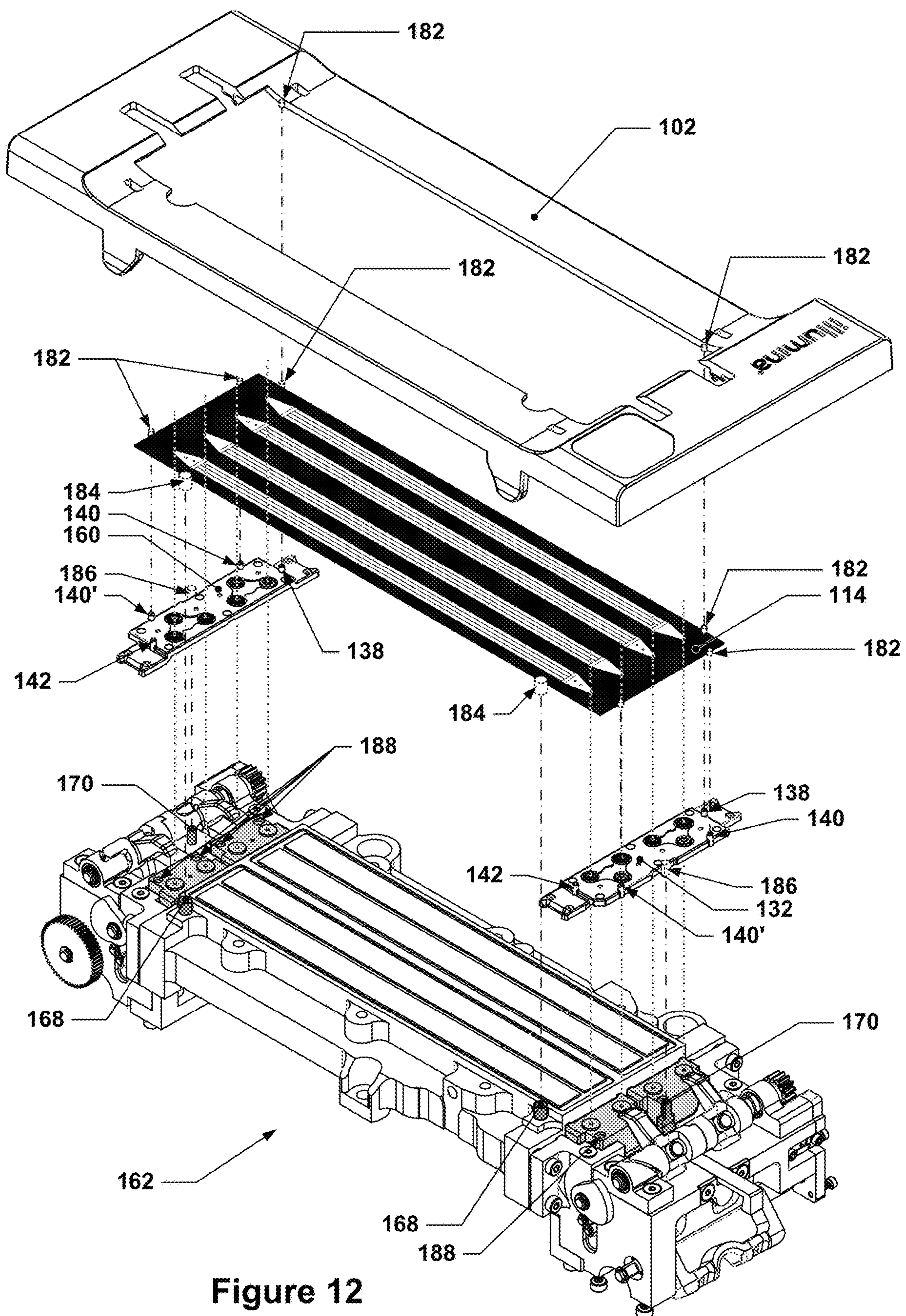


Figure 12



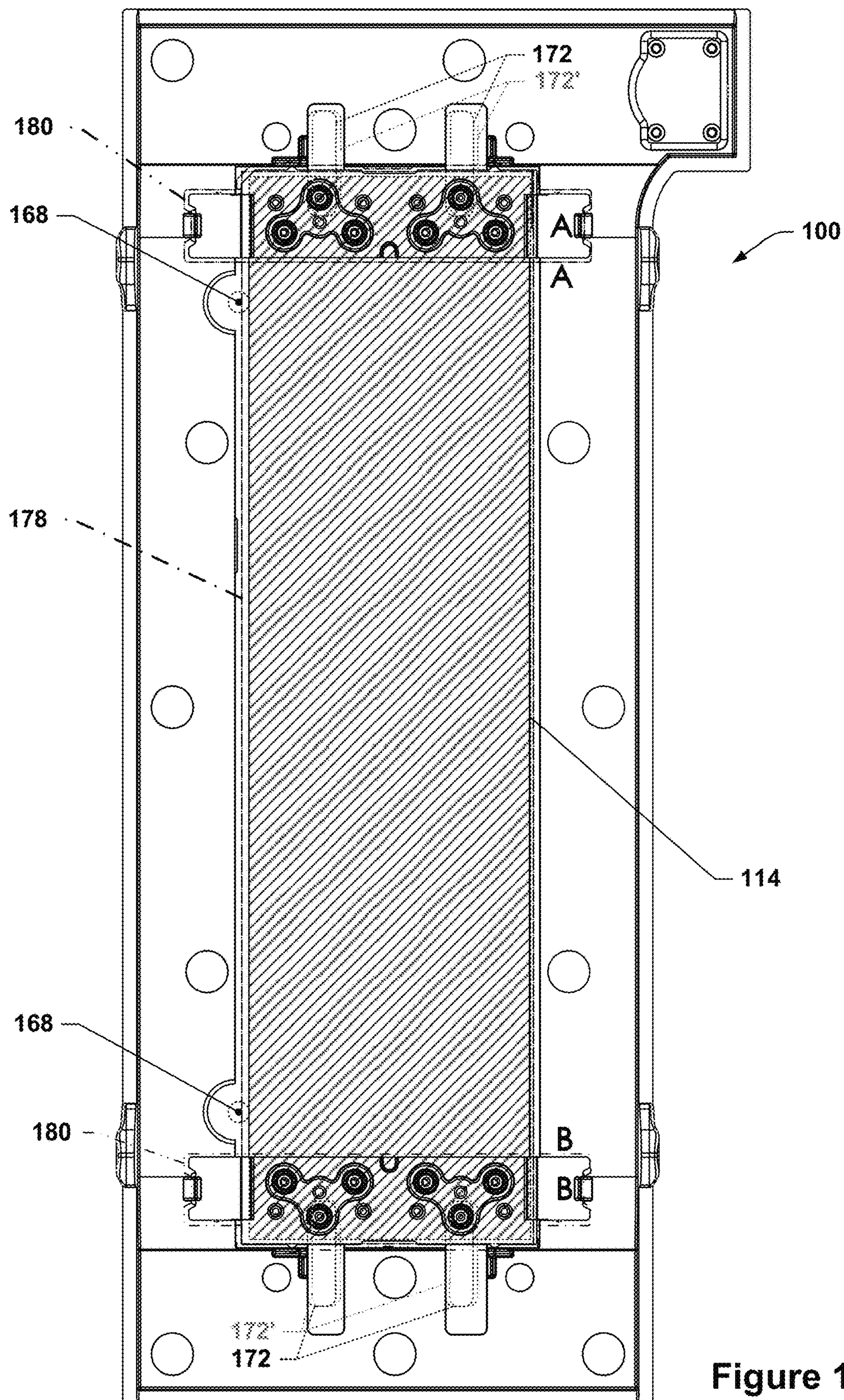


Figure 13



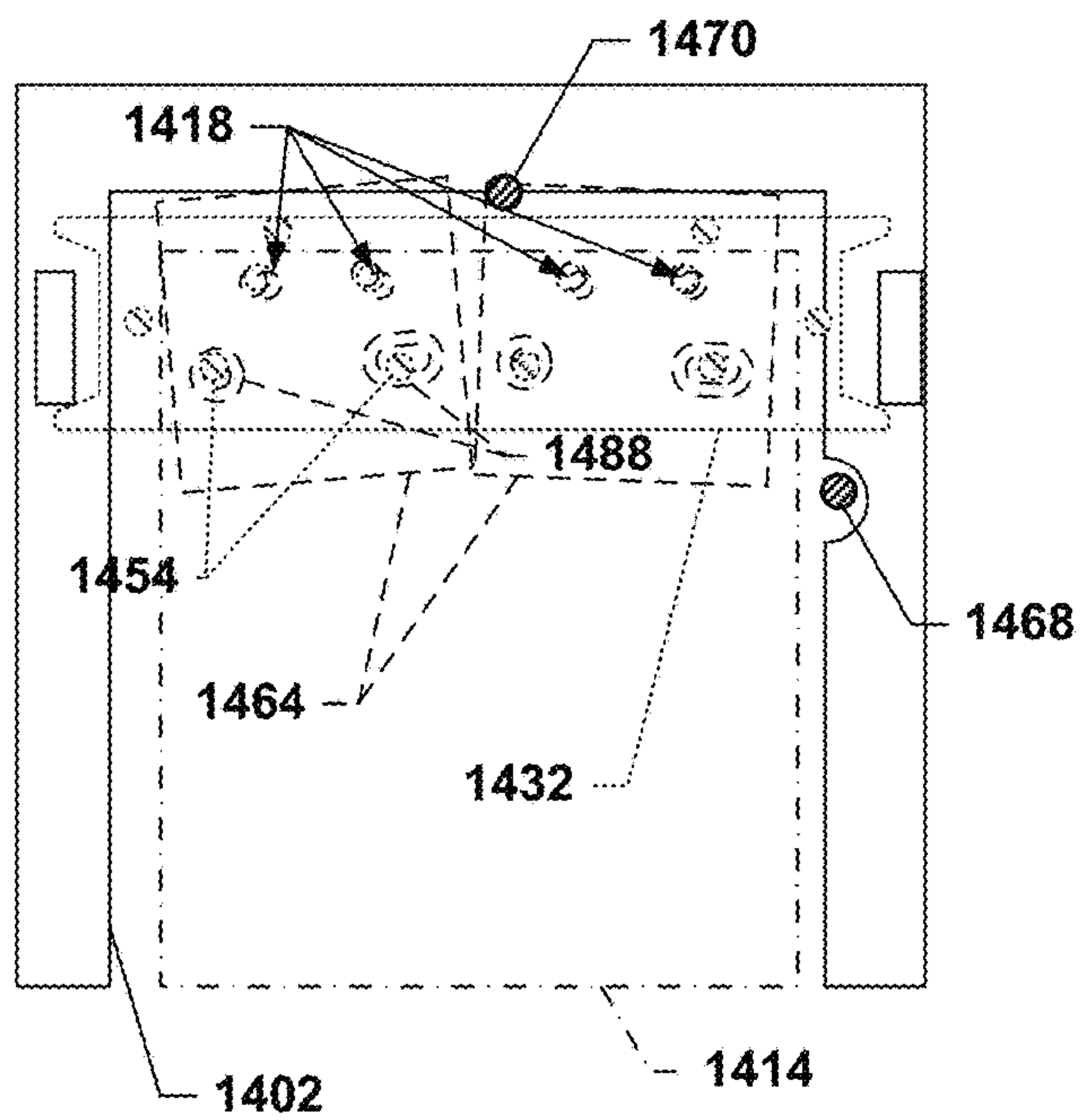


Figure 14

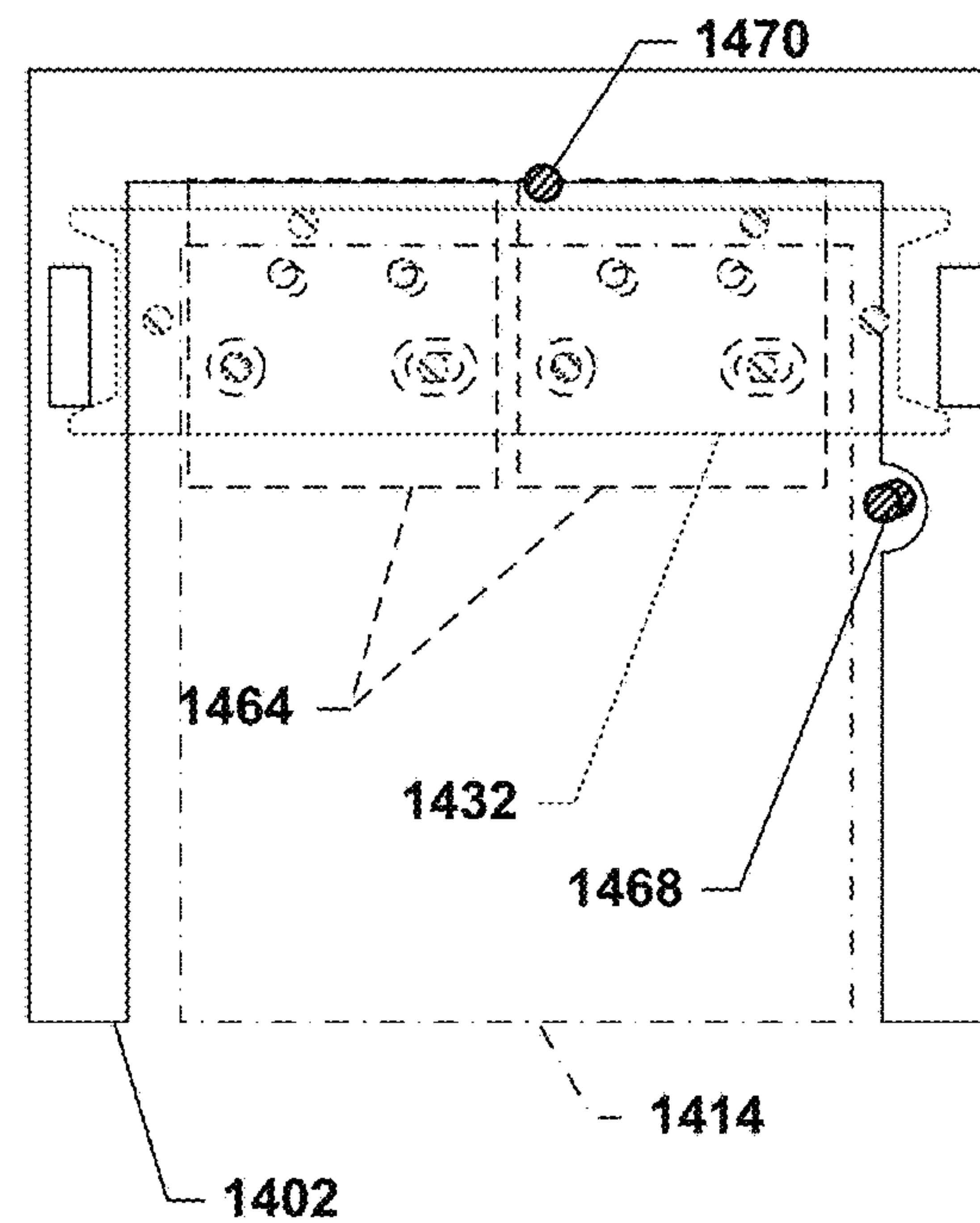


Figure 15

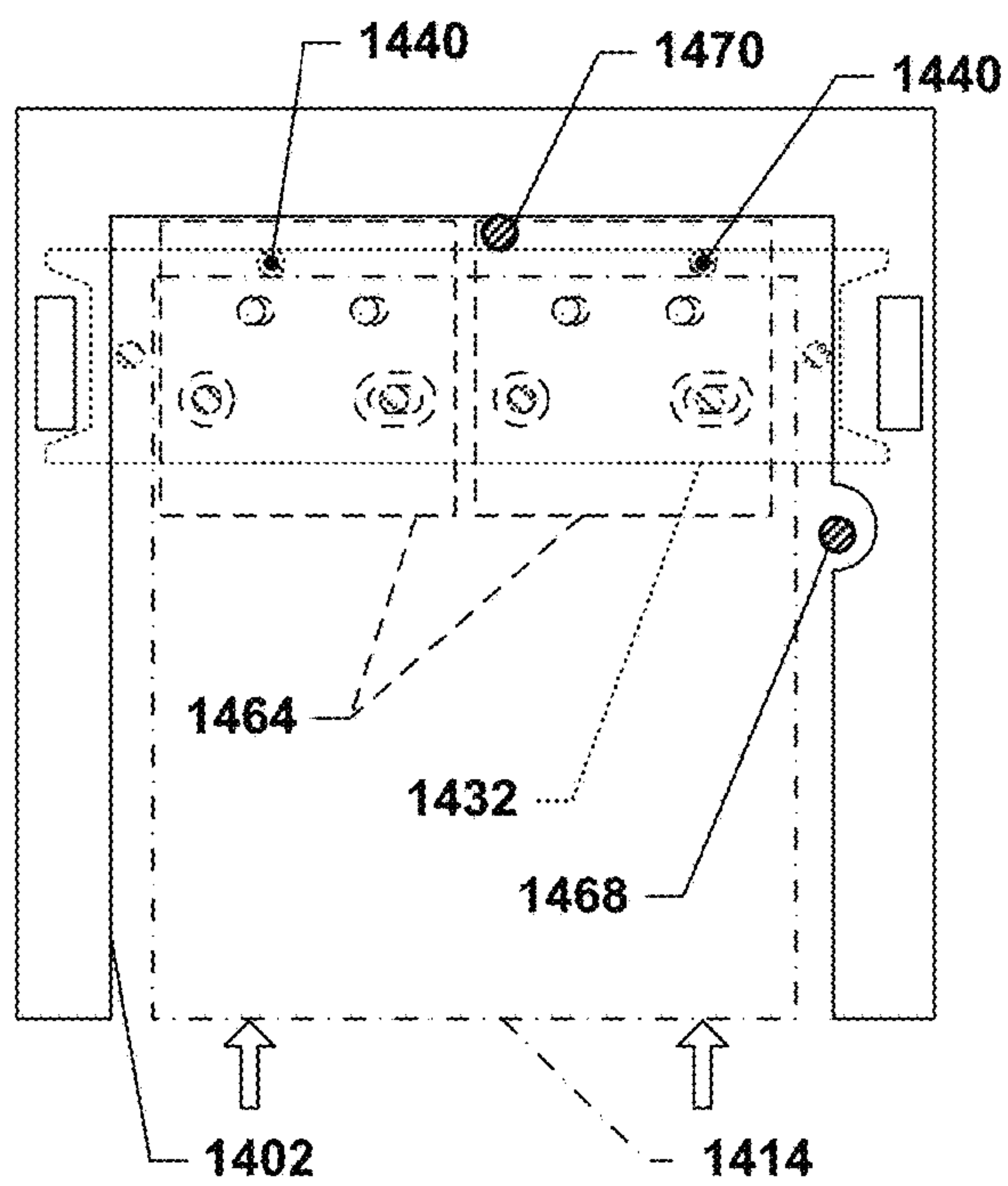


Figure 16

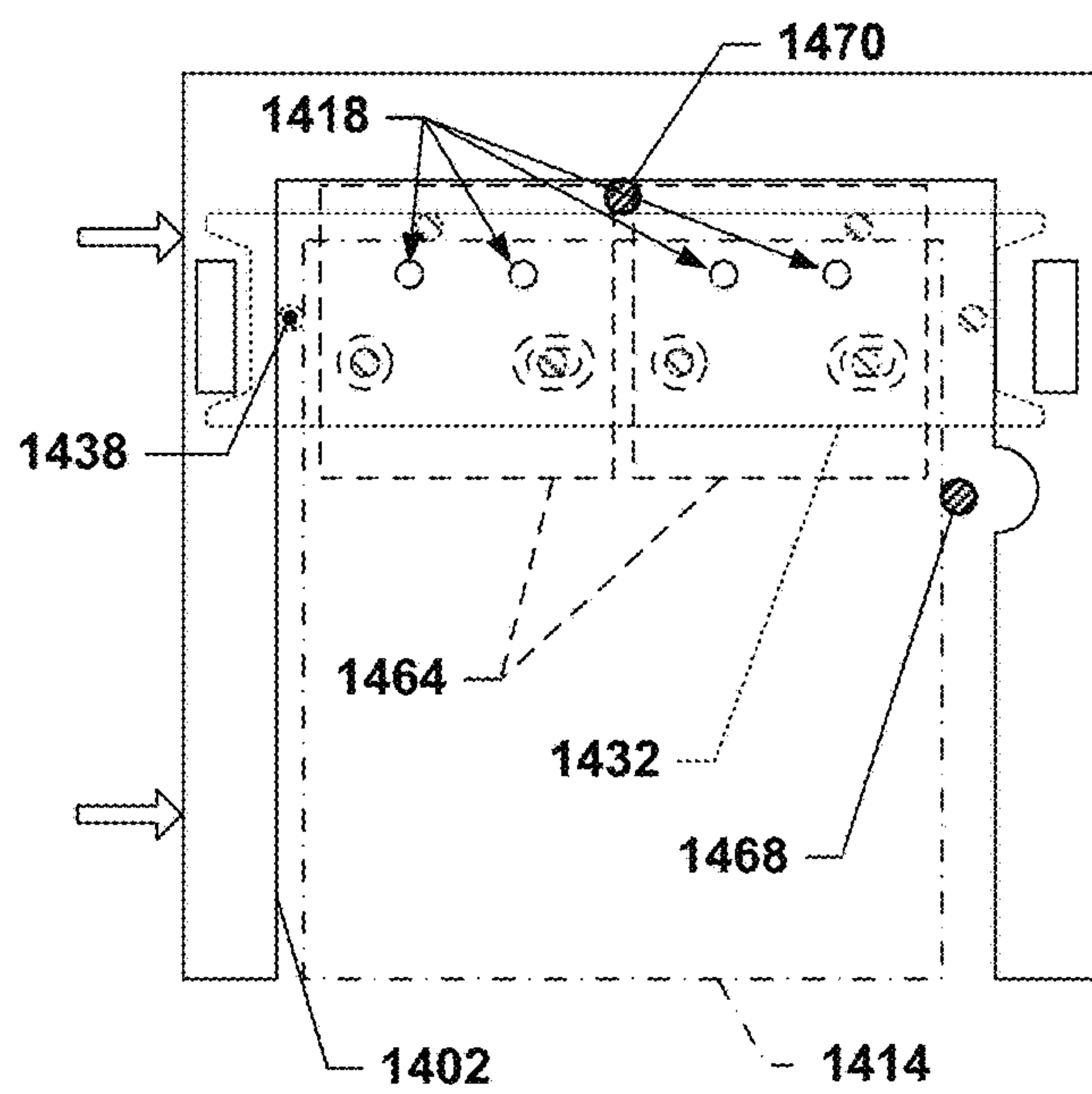


Figure 17



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**FLOWCELL CARTRIDGE WITH FLOATING  
SEAL BRACKET****CROSS-REFERENCE TO RELATED  
APPLICATION**

This application is a continuation application under 35 U.S.C. § 120 of U.S. patent application Ser. No. 18/167,836, filed Feb. 11, 2023, which is a divisional of U.S. patent application Ser. No. 16/777,881, filed Jan. 30, 2020, and issued as U.S. Pat. No. 11,577,253 on Feb. 14, 2023, which is itself a divisional application under 35 U.S.C. § 120 of U.S. patent application Ser. No. 16/436,485, filed Jun. 10, 2019, and issued as U.S. Pat. No. 10,549,282 on Feb. 4, 2020, and which is itself a continuation of U.S. patent application Ser. No. 15/841,109, filed Dec. 13, 2017, which issued as U.S. Pat. No. 10,357,775 on Jul. 23, 2019, and which claims benefit of priority to United Kingdom (GB) application 1704769.7, filed Mar. 24, 2017, and also claims benefit of priority under 35 U.S.C. § 119 (e) to U.S. Patent Application No. 62/441,927, filed Jan. 3, 2017, all of which are hereby incorporated by reference herein in their entireties.

**BACKGROUND**

Sequencers, e.g., genome sequencers, such as DNA sequencers or RNA sequencers, and other biological or chemical analysis systems may sometimes utilize microfluidic flowcells, such as may be provided by way of a glass plate having microfluidic flow channels etched therein. Such flowcells may be made as a laminated stack of layers, with the flow channels etched in one or more of the layers. In most flowcells, access to the flow channels within the flowcell may be provided by way of openings that pass through one or both of the outermost layers to reach the flow channels within.

Since it is difficult to decontaminate a flowcell after a sample has been flowed through it, it is common to replace the flowcell before analyzing a particular sample. As such, it is common for flowcells to be implemented using a cartridge-based approach to facilitate easy replacement of the flowcells.

**SUMMARY**

Details of one or more implementations of the subject matter described in this specification are set forth in the accompanying drawings and the description below. Other features, aspects, and advantages will become apparent from the description, the drawings, and the claims. Note that the relative dimensions of the following figures may not be drawn to scale unless specifically indicated as being scaled drawings.

In some implementations, an apparatus is provided that includes a frame, a microfluidic plate having one or more first fluidic ports in a first side, and a first support bracket that is attached to the frame such that the microfluidic plate is interposed between the first support bracket and the frame, the first support bracket floats relative to the microfluidic plate and the frame, the microfluidic plate and the frame float relative to one another, and a first side of the first support bracket faces towards the microfluidic plate. In such implementations, the first support bracket may include a first indexing feature that protrudes from the first side of the first support bracket and is proximate to a first edge of the microfluidic plate and may also include a second indexing

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feature that protrudes from the first side of the first support bracket and is proximate to a second edge of the microfluidic plate. The first support bracket may include a first gasket with at least one seal that is proud of the first side of the first support bracket and is positioned against the first side of the microfluidic plate, and the first indexing feature of the first support bracket and the second indexing feature of the first support bracket may contact the first edge and the second edge, respectively, of the microfluidic plate when the at least one seal of the first gasket is aligned with a corresponding at least one of the one or more first fluidic ports.

In some such implementations, the microfluidic plate may have a second side opposite the first side, the frame may have a first overlapping portion that overlaps, when viewed along a direction perpendicular to a major surface of the microfluidic plate, a first portion of the microfluidic plate that includes the second edge, the first overlapping portion may be proximate to the second side of the microfluidic plate, the first overlapping portion may have a first clamp arm slot having a first slot width in a direction parallel to the second edge, the second side of the microfluidic plate may be visible, e.g., to the unaided eye, through the first clamp arm slot, the apparatus may be to, or configured to be, interfaced with a receiver of an analysis device, the receiver having a first clamp arm that is movable from an unclamped position in which the first clamp arm does not press on the second side of the microfluidic plate and does not engage with the first clamp arm slot to a clamped position in which the first clamp arm presses on the second side of the microfluidic plate and engages with the first clamp arm slot, and the first slot width may be larger than a width of the first clamp arm in a direction parallel to the second edge and located within the first clamp arm slot when the first clamp arm is in the clamped position.

In some such implementations of the apparatus, the microfluidic plate may have a third edge opposite the first edge and a fourth edge opposite the second edge, the frame may have a second overlapping portion that overlaps, when viewed along the direction perpendicular to the major surface of the microfluidic plate, a second portion of the microfluidic plate that includes the fourth edge, the second overlapping portion may be proximate to the second side of the microfluidic plate, and the second overlapping portion may have a second clamp arm slot having a second slot width in a direction parallel to the fourth edge, the second side of the microfluidic plate may be visible through the second clamp arm slot, the receiver of the analysis device within which the apparatus is to be, or configured to be, interfaced may have a second clamp arm that is movable from an unclamped position in which the second clamp arm does not press on the second side of the microfluidic plate and does not engage with the second clamp arm slot to a clamped position in which the second clamp arm presses on the second side of the microfluidic plate and engages with the second clamp arm slot, and the second slot width may be larger than a width of the second clamp arm in a direction parallel to the fourth edge and located within the second clamp arm slot when the second clamp arm is in the clamped position.

In some implementations of the apparatus, there may be two first fluidic ports in the microfluidic plate, and the first gasket may include two seals, each seal having a through-hole passing through the first support bracket and aligned with a different one of the first fluidic ports when the first indexing feature of the first support bracket and the second



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indexing feature of the first support bracket contact the first edge and the second edge, respectively, of the microfluidic plate.

In some such implementations, the first gasket may include a support foot that is proud of the first side of the first support bracket and is positioned against the microfluidic plate, a first axis may be defined between center points of the two seals of the first gasket, the support foot of the first gasket may be offset by a first amount from the first axis along a second axis perpendicular to the first axis and parallel to the microfluidic plate, and the support foot of the first gasket may have an upper surface that contacts the microfluidic plate and is co-planar with upper surfaces of the two seals of the first gasket that are also in contact with the microfluidic plate. In some further such implementations of the apparatus, the support foot of the first gasket may not serve as a seal.

In some implementations of the apparatus, the first gasket may be co-molded into the first support bracket.

In some implementations of the apparatus, the first support bracket may have a second side that faces away from the first side of the first support bracket, and at least two first fluidic port indexing features may protrude from the second side of the first support bracket, each first fluidic port indexing feature to, or configured to, engage with a corresponding fluidic port indexing hole on a first fluidic port block of an analysis device to, or configured to, receive the apparatus.

In some implementations of the apparatus, the frame may include two opposing first retaining clips with opposing surfaces that face one another, the first support bracket may be positioned in between the two opposing first retaining clips, the opposing surfaces of the first retaining clips may be spaced apart by a first distance, and the portion of the first support bracket between the opposing surfaces of the first retaining clips may have a first width in a direction spanning between the opposing surfaces of the first retaining clips that is less than the first distance.

In some implementations of the apparatus, the first support bracket may include a third indexing feature that protrudes from the first side of the first support bracket and is proximate to a third edge of the microfluidic plate opposite the first edge of the microfluidic plate, and the microfluidic plate may be interposed between the first indexing feature of the first support bracket and the third indexing feature of the first support bracket.

In some implementations of the apparatus, the microfluidic plate may be rectangular and the first edge of the microfluidic plate may be orthogonal to the second edge of the microfluidic plate and the second edge of the microfluidic plate may be orthogonal to the third edge of the microfluidic plate.

In some implementations of the apparatus, the frame may have a substantially rectangular opening, the microfluidic plate may sit within the substantially rectangular opening, the substantially rectangular opening may have opposing side walls that face towards one another, and the first indexing feature of the first support bracket may be interposed between one of the opposing side walls of the substantially rectangular opening and the first edge of the microfluidic plate and the third indexing feature of the first support bracket may be interposed between the other opposing side wall of the opposing side walls of the substantially rectangular opening and the third edge of the microfluidic plate.

In some implementations of the apparatus, the substantially rectangular opening may have an opening width in a

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direction parallel to the second edge, a first indexing feature width may exist between furthest-apart portions of the surfaces of the first indexing feature of the first support bracket and the third indexing feature of the first support bracket that face the opposing side walls of the substantially rectangular opening, and the opening width minus the first indexing feature width may be less than the first distance minus the first width.

In some implementations, the microfluidic plate may further include one or more second fluidic ports on the first side and the apparatus may further include a second support bracket that is attached to the frame such that the microfluidic plate is interposed between the second support bracket and the frame, the second support bracket floats relative to the microfluidic plate and the frame, the microfluidic plate and the frame float relative to one another, and a first side of the second support bracket faces towards the microfluidic plate. In such implementations, the second support bracket may include a first indexing feature that protrudes from the first side of the second support bracket and is proximate to the first edge of the microfluidic plate, the second support bracket may include a second indexing feature that protrudes from the first side of the second support bracket and is proximate to a fourth edge of the microfluidic plate opposite the second edge of the microfluidic plate, the microfluidic plate may be interposed between the second indexing feature of the first support bracket and the second indexing feature of the second support bracket, the second support bracket may include a second gasket with at least one seal that is proud of the first side of the second support bracket and is positioned against the microfluidic plate, and the first indexing feature of the second support bracket and the second indexing feature of the second support bracket may contact the first edge and the fourth edge, respectively, of the microfluidic plate when the at least one seal of the second gasket is aligned with a corresponding at least one of the one or more second fluidic ports.

In some such implementations, the frame may include two opposing second retaining clips with opposing surfaces that face one another, the second support bracket may be positioned in between the two opposing second retaining clips, the opposing surfaces of the second retaining clips may be spaced apart by a second distance, and the portion of the second support bracket between the opposing surfaces of the second retaining clips may have a second width in a direction spanning between the opposing surfaces of the second retaining clips that is less than the second distance.

In some further such implementations, the second support bracket may include a third indexing feature that protrudes from the first side of the second support bracket and is proximate to the third edge of the microfluidic plate, and the microfluidic plate may be interposed between the first indexing feature of the second support bracket and the third indexing feature of the second support bracket.

In some additional such implementations, the frame may have a substantially rectangular opening, the microfluidic plate may have a third edge opposite the first edge, the microfluidic plate may sit within the substantially rectangular opening, the substantially rectangular opening may have opposing side walls that face towards one another and that define an opening width in a direction parallel to the second edge, the first indexing feature of the second support bracket may be interposed between one of the opposing side walls of the substantially rectangular opening and the first edge of the microfluidic plate and the third indexing feature of the second support bracket may be interposed between the other opposing side wall of the opposing side walls of the sub-



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stantially rectangular opening and the third edge of the microfluidic plate, the microfluidic plate may have a plate width in a direction spanning between the first indexing feature of the second support bracket and the third indexing feature of the second support bracket, a second indexing feature width may exist between furthest-apart portions of the surfaces of the first indexing feature of the second support bracket and the third indexing feature of the second support bracket that face the opposing side walls of the substantially rectangular opening, and the opening width minus the second indexing feature width may be less than the second distance minus the second width.

In some implementations, there may be two second fluidic ports in the microfluidic plate, and the second gasket may include two seals, each seal having a through-hole passing through the second support bracket and aligned with a different one of the second fluidic ports when the first indexing feature of the second support bracket and the second indexing feature of the second support bracket contact the first edge and the fourth edge, respectively, of the microfluidic plate.

In some implementations, the second gasket may include a support foot that is proud of the first side of the second support bracket and is positioned against the microfluidic plate, a third axis may be defined between center points of the two seals of the second gasket, the support foot of the second gasket may be offset by a second amount from the third axis along a fourth axis perpendicular to the third axis and parallel to the microfluidic plate, and the support foot of the second gasket may have an upper surface that contacts the microfluidic plate and may be co-planar with upper surfaces of the two seals of the second gasket that are also in contact with the microfluidic plate. In some such implementations, the support foot of the second gasket may not serve as a seal. In some alternative or additional such implementations, the second gasket may be co-molded into the second support bracket.

In some implementations, the second support bracket may have a second side that faces away from the first side of the second support bracket, and at least two second fluidic port indexing features may protrude from the second side of the first support bracket, each first fluidic port indexing feature to, or configured to, engage with a corresponding fluidic port indexing hole on a first fluidic port block of an analysis device to, or configured to, receive the apparatus.

These and other implementations are described in further detail with reference to the Figures and the detailed description below. Other features, aspects, and advantages will become apparent from the description, the drawings, and the claims. Note that the relative dimensions of the following figures may not be drawn to scale.

## BRIEF DESCRIPTION OF THE DRAWINGS

The various implementations disclosed herein are illustrated by way of example, and not by way of limitation, in the figures of the accompanying drawings, in which like reference numerals refer to similar elements.

FIG. 1 depicts an exploded isometric view of an example flowcell cartridge.

FIG. 2 depicts an exploded underside isometric view of the example flowcell cartridge of FIG. 1.

FIG. 3 depicts a front isometric view of the example flowcell cartridge of FIG. 1 in an unexploded state.

FIG. 4 depicts a rear isometric view of the example flowcell cartridge of FIG. 1 in an unexploded state.

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FIGS. 5 and 6 are diagrams illustrating how a seal can roll when the surfaces between which the seal is interposed are translated laterally.

FIGS. 7 and 8 are diagrams illustrating how a gasket with a support foot can prevent the rolling behavior illustrated in FIGS. 5 and 6.

FIG. 9 depicts an isometric view of a floating support bracket of the example flowcell cartridge of FIG. 1.

FIG. 10 depicts an underside isometric view of the floating support bracket of the example flowcell cartridge of FIG. 1.

FIG. 11 depicts an isometric view of an example receiver for the example flowcell cartridge of FIG. 1.

FIG. 12 depicts an exploded isometric view of the example receiver of FIG. 11 and the example flowcell cartridge of FIG. 1.

FIG. 13 depicts a plan view of the example flowcell cartridge of FIG. 1.

FIGS. 14 through 17 depict various stages of component alignment that may occur during clamping of an example flowcell cartridge.

FIGS. 1 through 4 and 9 through 13 are drawn to scale within each Figure, although the scale of the depicted embodiments may vary from Figure to Figure.

## DETAILED DESCRIPTION

The present inventors have conceived of new designs for a flowcell cartridge, such as may be used in chemical and biological analysis systems that utilize microfluidic flow structures contained within a glass plate structure. These concepts are discussed herein with respect to the following Figures, although it will be appreciated that these concepts may be implemented in cartridge designs other than the specific example shown, and that such other implementations would still potentially fall within the scope of the claims.

FIG. 1 depicts an exploded isometric view of an example flowcell cartridge. In FIG. 1, the flowcell cartridge 100 has a frame 102 that may, for example, be made of molded plastic or other, durable material. The frame may provide a support structure for supporting a glass plate (or a plate of other material, e.g., acrylic or other plastic), such as glass plate 114 that contains microfluidic flow structures; this plate may also be referred to herein as a microfluidic plate. In this example, the glass plate, which has a first edge 122, a second edge 124, a third edge 126, and a fourth edge 128, includes four sets of multiple, parallel microfluidic flow channels that extend along directions parallel to the long axis of the glass plate, e.g., along axes that are parallel to the first edge 122 and/or the third edge 126. To the extent applicable, the terms “first,” “second,” “third,” etc. (or other ordinal indicators) herein are merely employed to show the respective objects described by these terms as separate entities and are not meant to connote a sense of chronological order, unless stated explicitly otherwise herein. The first edge 122 and the third edge 126 may be generally orthogonal to the second edge 124 and the fourth edge 128 in some implementations, but may be other orientations in other implementations. As can be seen in FIG. 2, which depicts an exploded underside isometric view of the example flowcell cartridge of FIG. 1, each set of microfluidic flow structures may terminate in one or more first fluidic ports 118 and one or more second fluidic ports 120. The first and second fluidic ports 118 and 120 may be located in a first side 116 of the glass plate 114, although other implementations may only include the first fluidic ports 118 or the second fluidic ports



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120 on the first side 116. The frame 102 may have a substantially rectangular opening (or opening of another shape) 104 that is sized to receive the glass plate 114; the rectangular opening 104 may include opposing side walls 106 that are in close proximity to the first edge 122 and the third edge 126 of the glass plate 114 when the cartridge is fully assembled. As used herein, the term “substantially rectangular” is use to refer to an opening that has an overall rectangular shape, although there may be various features or discontinuities in the overall shape, such as the semi-circular notches along one side wall of the depicted rectangular opening, or the clamp arm slots along the short edges of the rectangular opening 104. The opposing side walls 106 may be spaced apart by an opening width 195 to allow the first support bracket 132 and the second support bracket 160, and thus the glass plate 114, to float within the rectangular opening 104 for at least some range of movement, e.g., about 1 mm to about 2 mm or less.

The glass plate 114 may be held in place in the cartridge 100 through the use of one or more support brackets, such as a first support bracket 132 and a second support bracket 160. In this discussion, only the features of the first support bracket 132 are discussed in detail, although it is readily apparent from the Figures that the second support bracket 160, which may or may not be identical to the first support bracket 132, is at least structurally similar to the first support bracket 132 and may operate in a similar manner.

The first support bracket 132 may have a first side 134 (see FIG. 1) and a second side 136 (see FIG. 2). The first side 134 may face towards the glass plate 114 and may have a first indexing feature 138, e.g., a molded pin or post, that extends away from the first side 134 and that is at least long enough that the side of the first indexing feature 138 that faces towards the glass plate 114 may contact the glass plate 114 when the cartridge is fully assembled. The first indexing feature 138 may be positioned on the first support bracket 132 such that the first indexing feature 138 is proximate to, or contacting, the first edge 122 of the glass plate 114 when the cartridge is fully assembled. The first support bracket 132 may also have one or more second indexing features 140 (an additional second indexing feature 140' is also shown in FIG. 1) that may be similar to the first indexing feature 138 except that each second indexing feature 140 may be positioned on the first support bracket 132 such that the second indexing feature 140 is proximate to, or physically contacts, the second edge 124 of the glass plate 114. The first support bracket 132 may also include a third indexing feature 142, which may be positioned on an opposite end of the first support bracket 132 from the first indexing feature 138. The first indexing feature 138 and the third indexing feature 142, if used, may be separated from one another by a first float gap 156, which may be sized to be slightly larger than the plate width 130 so as to allow the glass plate 114 to “float” within the confines of the first indexing feature 138 and the third indexing feature 142. The furthest-apart surfaces of the first indexing feature 138 and the third indexing feature 142 may similarly define a first indexing feature width 157. The opening width 195 may be wider than the first indexing feature width 157 so that the first support bracket 132 may float laterally between the opposing side walls 106 of the rectangular opening 104.

The first support bracket may also include one or more first gaskets 144, which may include one or more seals 146 (each first gasket 144, in this example, includes two seals 146, each positioned so as to interface with a different first fluidic port 118). The first gaskets 144 may, for example, be insertable into the first support bracket 132 or may, in some

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implementations, be co-molded with the first support bracket 132 (in the latter case, the first gaskets 144 and the first support bracket 132 may, in effect, be treated as a single component). The seals may be proud of the first side 134 and, optionally, the second side 136 of the first support bracket so that they may compress against the glass plate 114 and, as discussed later herein, a fluidic port block, respectively. In some implementations, the seal may not be proud of the second side 136 of the first support bracket, e.g., if the fluidic port block that faces the second side 136 when the cartridge is installed in an analysis device has a raised boss that may engage with the seal.

The first gasket 144 may also include a support foot 148, which may be provided to prevent or mitigate “rolling” of the first gasket 144 about an axis passing through the centers of the seals 146 when the first support bracket 132 is translated in a direction parallel to the major surface of the glass plate 114 while the seals 146 are in contact with the glass plate 114. To this end, the support foot 148 may be offset from a first axis 150 spanning between the centers of the seals 146 of the first gasket 144 along a second axis 152 perpendicular to the first axis 150 by some amount so as to provide a moment arm to resist such rolling behavior. The support foot 148 and the seals 146 may all be designed to have contact surfaces that contact the glass plate 114 in concert when the glass plate 114 is brought into contact with the first gasket 144. These contact surfaces may all be parallel to one another to ensure that when the contact surface of the support foot 148 is in contact with the glass plate 114, the contact surface(s) of the seal(s) 146 are also in good, i.e., not having any misalignment gaps, contact with the glass plate 114. In the example cartridge shown, each support bracket includes two first gaskets, although they may be referred to as second gaskets, third gaskets, etc., in the interests of reducing confusion, if needed. It is also understood that the support foot 148, while appearing similar to the seals 146, may actually not provide any “sealing” characteristics at all—it may be present solely for the purposes of preventing or mitigating “rolling.”

FIGS. 5 and 6 are diagrams illustrating how a seal can roll when the surfaces between which the seal is interposed are translated laterally. In FIG. 5, a glass plate 514 is offset from a fluidic port block 564, and a support bracket 532 with a gasket 544 is interposed between them. The gasket 544 has a seal 546 that is aligned with a fluidic port 518' in the fluidic port block 564, but that is misaligned somewhat with a fluidic port 518 in the glass plate 514. As can be seen in FIG. 6, when the glass plate 514 is slid sideways so that the fluidic port 518 is aligned with the seal 546, friction between the seal 546 and the glass plate 514/fluidic port block 564 may cause the seal 546 to not slide a commensurate distance—as a result, the gasket 544 and the support bracket 532 may tilt or roll slightly, resulting in gaps 594 appearing between the seal 546 and the glass plate 514/fluidic port block 564. This is, of course, undesirable, as it causes leakage.

FIGS. 7 and 8 are diagrams illustrating how a gasket with a support foot can prevent the rolling behavior illustrated in FIGS. 5 and 6. As can be seen, the gasket 544 has been extended to the right and a support foot 748 has been added to the gasket 544. When the glass plate 514 is slid to the left, as in FIG. 6, the support foot 748 introduces a counter-moment to any potential rolling moment caused by friction between the seal 546 and the glass plate 514/fluidic port block 564. This prevents the formation of the gaps 594 and keeps the seal 546 in good contact with the surfaces it seals.

The first support bracket 132 may snap into two opposing first retaining clips 108 (only one is visible in FIG. 2, as the



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other is obscured by other features of the frame **102**—however, there are corresponding second retaining clips visible on the opposite end of the frame **102** that are configured similarly but at a different location). The first retaining clips **108** may have opposing surfaces **110** that are separated from one another by a first distance **112**. The first distance may be greater than a first width **158** of the first support bracket **132**, thereby allowing the first support bracket **132** to float laterally by a small amount when snapped into the first retaining clips **108**. In some implementations, the amount of float between the first support bracket **132** and the opposing side walls **106**, i.e., the opening width **195** minus the first indexing feature width **157**, may be smaller than the amount of float between the first support bracket **132** and the retaining clips **108**, i.e., the first distance **112** minus the first width **158**. Similar relationships may exist for the second support bracket **160**.

FIG. **3** depicts a front isometric view of the example flowcell cartridge of FIG. **1** in an unexploded/assembled state. FIG. **4** depicts a rear isometric view of the example flowcell cartridge of FIG. **1** in an unexploded/assembled state. As can be seen, the glass plate **114** is held in place within the frame **102** by the first support bracket **132** and the second support bracket **160**, which, in turn, are held in place by the first retaining clips **108** and second retaining clips, respectively. The frame may have a first overlapping portion **196** and a second overlapping portion **196'** (see FIG. **2**) that overlap with a corresponding first portion **197** and second portion **197'** (see FIG. **1**) of the glass plate **114**. The first portion **197** may include the second edge **124**, and the second portion **197'** may include the fourth edge **128**. The overlapping portions **196/196'** may prevent the glass plate **114** from falling out of the front of the frame **102**, e.g., the glass plate **114** may be sandwiched between the overlapping portions **196/196'** and the first/second support brackets **132/160**. The glass plate **114** may still, however, be free to float within the frame to some degree.

FIG. **9** depicts an isometric view of the first support bracket **132** of the example flowcell cartridge **100** of FIG. **1**. FIG. **10** depicts an underside isometric view of the first support bracket **132** of the example flowcell cartridge **100** of FIG. **1**. In addition to the first indexing feature **138**, the second indexing feature(s) **140**, and possibly the third indexing feature **142**, the first support bracket **132** may also include first fluidic port indexing features **154** on the second side **136** of the first support bracket **132** (the second support bracket **160** may have corresponding second fluidic port indexing features as well). As can be seen, the first support bracket has portions that extend beyond the first width **158**, e.g., the small “teeth” that are located at the four outermost corners of the first support bracket **132**. These teeth may engage with the first retaining clips **108** and may allow the first support bracket **132** to also float along an axis parallel to the first edge **122** by some limited amount.

In this example cartridge, the glass plate **114** may float with respect to the support brackets **132** and **160**, and the support brackets **132** and **160**, in turn, may float with respect to the frame **102**. Thus, there are two tiers of floating components in the example cartridge. The combination of these different tiers of floating components, as well as the various indexing features provided, allow for the glass plate **114** and the seals **146** to be properly aligned with each other and with ports on floating manifold blocks located on equipment that receives the cartridge **100**.

FIG. **11** depicts an isometric view of an example receiver for the example flowcell cartridge of FIG. **1**. As seen in FIG. **11**, a receiver **162** may be provided; the receiver may be a

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subcomponent of a larger analysis device that utilizes the cartridge **100**. The receiver **162** may include a chuck **176**, against which the glass plate **114** may be drawn, e.g., by a vacuum, during analysis operations. The receiver **162**, in this example, may include a pair of first fluidic port blocks **164** and an opposing pair of second fluidic port blocks **166**. The first fluidic port blocks **164** and the second fluidic port blocks **166** may be configured to float slightly in directions at least parallel to the upper surface of the chuck **176** (and possibly also in directions perpendicular to the upper surface of the chuck **176**). The ends of the receiver **162** may include, for example, a clamping mechanism that may serve to clamp the glass plate **114** against the chuck **176**. Such clamping mechanisms may, for example, have clamp arms **172** that may rotate downwards and contact the upper surface of the glass plate **114** of the cartridge **100** when the cartridge **100** is installed. The receiver **162** may also include indexing features that are located so as to engage with the support brackets and glass plate **114** of the cartridge **100** when the cartridge **100** is installed. For example, lateral indexing pins **168** may be placed such that the glass plate **114** contacts the lateral indexing pins **168** when the glass plate **114** is translated laterally along the short axis of the chuck **176**, and longitudinal indexing pins **170** may be positioned so as to contact the support brackets of the cartridge **100** when, for example, one of the longitudinal indexing pins **170** is moved towards the other longitudinal indexing pins **170**. In this example, the longitudinal indexing pin **170** on the left is fixed in space relative to the receiver **162**, whereas the other longitudinal indexing pin **170** is configured to slide along an axis parallel to the long axis of the chuck **176**. The sliding longitudinal indexing pin **170** may be sprung so as to be biased towards the other longitudinal indexing pin **170**. The interaction of the various indexing features is explained in more detail below, with respect to FIG. **12**.

FIG. **12** depicts an exploded isometric view of the example receiver of FIG. **11** and the example flowcell cartridge of FIG. **1**. In this example, the cartridge **100** has been shown in an exploded view, although the various components that form the cartridge would be fully assembled, per FIG. **3**, prior to the cartridge **100** being placed in the receiver **162**.

When the cartridge **100** is laid on top of the receiver **162**, the clamp arms **172** may rotate downward and engage with the top side of the glass plate **114**. The clamp arms **172** may also, as they pivot, translate along their rotational axes towards the lateral indexing pins **168** such that the sides of the clamp arms **172** engage with the sides of the rectangular notches or clamp arm slots **198**, thereby causing the entire frame **102** to translate along the same axis as well. For example, the clamp arm slots **198** may be sized, e.g., with clamp arm widths **173** in a direction parallel to the second edge **124** that are less than the widths of the clamp arm slots **198** in the same direction, to allow the clamp arms **172** to swing through the clamp arm slots **198** freely and, during lateral translation of the clamp arms **172**, press against the sides of the clamp arm slots **198** facing away from the lateral indexing pins **168**, thereby pushing the frame **102** towards the lateral indexing pins **168**. During this lateral sliding motion, the frame **102** will (if not already in such a state) come into contact with the first indexing feature **138** on the first support bracket **132** (and a corresponding first indexing feature on the second support bracket **160**) at indexing feature contact points **182** located along one of the opposing side walls **106**. As the frame **102** continues to be translated towards the lateral indexing pins **168**, the glass plate **114** will eventually come into contact with both the lateral indexing



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pins **168** and the first indexing features **138** (see lateral indexing pin contact points **184** and the indexing feature contact points **182** along the first edge **122** of the glass plate **114**). Eventually, the first indexing features **138** will be sandwiched between the frame **102** and the glass plate **114** (which is pressed against the lateral indexing pins **168**), thereby locating the first support bracket **132** and the second support bracket **160** firmly in space in the lateral direction, i.e., perpendicular to the long axis of the chuck **176**. This aligns the seals on the first support bracket **132** and the second support bracket **160** with the corresponding first fluidic ports **118** and the corresponding second fluidic ports **120**, respectively, on the glass plate **114**.

Subsequent to, after, or in concert with the translation of the frame **102** towards the lateral indexing pins **168**, the longitudinal indexing pins **170** may be caused to move towards one another (one or both may move), thereby contacting the facing edges of the first support bracket **132** and the second support bracket **160** and pushing the first support bracket **132** and the second support bracket **160** towards one another. As the first support bracket **132** and the second support bracket **160** move towards one another, the glass plate **114** may come into contact with the second indexing features **140** (and **140'**, if present) on the first support bracket **132** and the second support bracket **160**. The first support bracket **132** and the second support bracket **160** may thus become aligned with the glass plate **114** and, consequently, the first fluidic ports **118** and the second fluidic ports **120**.

After or during such plate alignment, the fluidic port blocks **164**, **166** may be raised so that the first fluidic port indexing features **154** (and corresponding second fluidic port indexing features on the second support bracket **160**) may be inserted into corresponding alignment holes **188** on the first fluidic port block **164** and the second fluidic port block **166**. As the fluidic port block rises, the first fluidic port indexing features **154** and the second fluidic port indexing features may engage with the corresponding alignment holes **188** and force the first fluidic port blocks **164** and the second fluidic port blocks **166** into alignment with the first support bracket **132** and the second support bracket **160**, respectively. This, in turn, ensures that the corresponding seals **146** on the respective support brackets **132**, **160** line up with the fluidic ports on the first fluidic port blocks **164** and the second fluidic port blocks **166**, respectively.

Thus, the cartridge **100** may have multiple levels of floating components that engage with different sets of indexing features/pins in the cartridge **100** and located on the receiver **162** and are moved into precisely aligned positions that cause the fluidic ports, seals, and port block ports to line up, e.g., such that the centerlines of the fluidic ports, seals, and port block ports are, in some implementations, within less than about 0.05 mm of one another, thereby ensuring a high-quality liquid-tight seal. At the same time, some implementations of the cartridge may feature additional features in the floating brackets, e.g., support feet, that may prevent rolling behavior of the seal, thereby ensuring the integrity of any sealed connections. Some of the floating components, e.g., the support brackets, may also act to retain other floating components, e.g., the glass plate, in a manner that prevents stressing the glass plate due to thermal expansion mismatches between the glass plate and the cartridge frame, minor flexure of the cartridge frame, and so forth.

The floating behavior of the various components in the cartridge **100** may be better understood with reference to FIG. **13**, which depicts a plan view of the example flowcell cartridge of FIG. **1**. For reference purposes, the lateral

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indexing pins **168** are shown as dotted circles and the outlines of the clamp arms **172** are shown as dotted, rounded rectangles, but the remainder of the components shown are part of the cartridge **100**. The clamp arms **172** are shown in both an “engaged” position (black line font) in which they are engaged with and pressed against the sides of the clamp arm slots **198** (see FIG. **2**) and a non-engaged position (grey line font), which may be their position prior to translating laterally. The glass plate **114** maybe able to move laterally by an amount relative to the frame **102** that is limited by the first and second indexing features **138** and **142**, respectively **11**. The first and second support brackets may be able to move laterally (as well as longitudinally) by a lesser amount, as is shown by the bracket float envelopes **180**. For example, the first and second support brackets may be able to float laterally by a distance of X, which may be the opening width **195** minus the first indexing feature width **157**, relative to the frame, and the glass plate **114** may be able to float laterally by a distance of Y, which may be the first float gap **156** minus the plate width **130**, relative to the first and second support brackets **132** and **160**. In some such implementations, Y may be less than X-however, the glass plate **114** may still float by a larger amount relative to the frame **102** than the first and second support brackets **132** and **160** since the glass plate **114** has a total overall float relative to the frame **102** of X+Y. This may allow for considerable adjustment in the positioning of the glass plate.

An example alignment sequence is reviewed in FIGS. **14** through **17**, which depict various stages of component alignment that may occur during clamping of an example flowcell cartridge. In FIG. **14**, the frame **1402** (shown in solid lines) of a flowcell cartridge is lowered onto a receiver with two floating fluidic port blocks **1464** (shown in dashed lines). As can be seen, the fluidic port blocks **1464** are slightly askew due to the fact that both are “floating.” Also visible in FIG. **14** is the outline of a support bracket **1432** (dotted lines) and a glass plate **1414** (dash-dot-dash lines). There are four instances of fluidic ports **1418** across the glass plate **1414**. As can be seen, at each fluidic port **1418**, there are corresponding features belonging to the support bracket (dotted circles) and fluidic port blocks (dashed lines). These correspond, for example, to the holes in the seals **146** and to the ports in the fluidic port blocks **1464**. As is evident, there is some alignment between these three separate fluidic flow features at each location, but the alignment is far from ideal, resulting in differently-configured apertures at each location which may cause imbalances in fluid flow.

In FIG. **15**, the support bracket **1432** has been fully engaged with the fluidic port blocks **1464** so that fluidic port indexing features **1454** (see FIG. **14**) are fully inserted into alignment holes **1488** (also see FIG. **14**). The alignment holes **1488**, for example, may be countersunk and the fluidic port indexing features **1454** may have conical or rounded tips so that they may engage with one another even if somewhat misaligned; as the fluidic port indexing features **1454** are more fully engaged with the alignment holes **1488**, the countersink portion may narrow and force the fluidic port indexing features **1454** to move towards the center of the alignment holes **1488**. As can be seen, one of the alignment holes **1488** for a given fluidic port block **1464** may be circular, thereby providing both X and Y location constraints, whereas the other may be obround to provide a single degree of constraint, e.g., along only the Y axis, as this may be all that is needed in one implementation to prevent rotation about the other alignment hole **1488**. It is to be recognized that the alignment holes **1488** and the fluidic port indexing features **1454** may also be swapped, i.e., the



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alignment holes **1488** may be located on the support bracket **1432**, and the fluidic port indexing features **1454** may be located on the fluidic port block **1464**.

Returning to FIG. **15**, the interfacing of the cartridge with the fluidic support blocks **1464** causes the fluidic port blocks **1464** to come into alignment with each other as well as with the support bracket **1432**. Consequently, the ports on the fluidic port blocks **1464** are now precisely aligned with the holes, e.g., the seals, on the support bracket **1432**. However, the holes/seals on the support bracket **1432** are not yet aligned with the fluidic ports **1418** on the glass plate.

In FIG. **16**, the glass plate **1414** has been moved upwards to contact second indexing features **1440** on the support bracket **1432**; this contact and the upward movement of the glass plate **1414** causes the support bracket **1432** to move upwards until it contacts longitudinal indexing pin **1470**, thus firmly locking the support bracket **1432** in place in the vertical direction (with respect to the Figure orientation; in reality, this is more accurately called the longitudinal direction)—this aligns the fluidic ports **1418** in the glass plate **1414** with the corresponding holes/seals in the support bracket **1432** in the vertical direction.

Finally, in FIG. **17**, the frame **1402** may be pushed towards the lateral indexing pin **1468**. This causes the inside edge of the frame **1402** to contact first indexing feature **1438**, which causes the support bracket **1432**, in turn, to move towards the lateral indexing pin **1468** until the first indexing feature **1438** also contacts the glass plate **1414** and pushes the opposite side of the glass plate **1414** into contact with the lateral indexing pin **1468**. As can be seen, the first fluidic ports **1418** and the respective seal holes and fluidic port block holes are completely aligned, thereby ensuring a consistently-sized flow aperture and proper seal alignment.

The term “about” used throughout this disclosure, including the claims, is used to describe and account for small fluctuations, such as due to variations in processing. For example, unless otherwise specified herein in a particular context, they can refer to less than or equal to  $\pm 5\%$ , of the specified value or value equivalent to the specified relationship, such as less than or equal to  $\pm 2\%$ , such as less than or equal to  $\pm 1\%$ , such as less than or equal to  $\pm 0.5\%$ , such as less than or equal to  $\pm 0.2\%$ , such as less than or equal to  $\pm 0.1\%$ , such as less than or equal to  $\pm 0.05\%$ .

As noted earlier, any use of ordinal indicators, e.g., (a), (b), (c) . . . or the like, in this disclosure and claims is to be understood as not conveying any particular order or sequence, except to the extent that such an order or sequence is explicitly indicated. For example, if there are three steps labeled (i), (ii), and (iii), it is to be understood that these steps may be performed in any order (or even concurrently, if not otherwise contraindicated) unless indicated otherwise. For example, if step (ii) involves the handling of an element that is created in step (i), then step (ii) may be viewed as happening at some point after step (i). Similarly, if step (i) involves the handling of an element that is created in step (ii), the reverse is to be understood.

It is also to be understood that the use of “to,” e.g., “the apparatus is to be interfaced with a receiver of an analysis device,” may be replaceable with language such as “configured to,” e.g., “the apparatus is configured to be interfaced with a receiver of an analysis device”, or the like.

It should be appreciated that all combinations of the foregoing concepts (provided such concepts are not mutually inconsistent) are contemplated as being part of the inventive subject matter disclosed herein. In particular, all combinations of claimed subject matter appearing at the end of this disclosure are contemplated as being part of the

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inventive subject matter disclosed herein. For the sake of brevity, many of those permutations and combinations will not be discussed and/or illustrated separately herein.

What is claimed is:

1. A microfluidic cartridge comprising:

a frame;

a microfluidic plate positioned within the frame, wherein the microfluidic plate floats relative to the frame, the microfluidic plate comprising a first side, a first edge, and a plurality of first fluidic ports located in the first side;

a support bracket positioned within the frame, wherein the support bracket floats relative to the microfluidic plate and the frame, the support bracket comprising:

a plurality of seals supported by the support bracket, each seal of the plurality of seals positioned to interface with a corresponding first fluidic port of the plurality of first fluidic ports, and

a plurality of alignment holes corresponding to a first plurality of indexing features located on an analysis device, the plurality of alignment holes configured to align the plurality of seals with corresponding analysis device ports located on the analysis device; and

wherein the frame includes a plurality of apertures proximate the first edge of the microfluidic plate, the plurality of apertures corresponding to a second plurality of indexing features located on the analysis device, and wherein the second plurality of indexing features engage the microfluidic plate and the first plurality of indexing features engage the plurality of alignment holes to align the plurality of first fluidic ports, the plurality of seals, and the second fluidic ports when the microfluidic cartridge is installed in the analysis device.

2. The microfluidic cartridge of claim 1, further comprising a plurality of teeth extending from the support bracket, wherein the plurality of teeth engage with the frame and partially constrain movement of the support bracket in a direction parallel to the first edge and relative to the frame.

3. The microfluidic cartridge of claim 1, wherein each seal of the plurality of seals has a through-hole passing through the seal to fluidically connect the plurality of first fluidic ports to the analysis device ports.

4. The microfluidic cartridge of claim 3, wherein the plurality of seals is received in the support bracket.

5. The microfluidic cartridge of claim 3, wherein the plurality of seals is co-molded with the support bracket.

6. The microfluidic cartridge of claim 1, further comprising a plurality of retaining clips that hold the microfluidic plate and the support bracket within the frame.

7. The microfluidic cartridge of claim 6, wherein:

the plurality of retaining clips comprises at least two opposing retaining clips spaced apart by a distance, the microfluidic plate has a width in a direction spanning between the opposing retaining clips that is less than the distance.

8. The microfluidic cartridge of claim 1, wherein the plurality of alignment holes comprises an alignment hole having a countersink portion that narrows to direct a corresponding indexing feature of the second plurality of indexing features toward the center of the alignment hole.

9. The microfluidic cartridge of claim 1, wherein the plurality of alignment holes comprises a circular alignment hole to constrain movement of the support bracket along perpendicular axes of the microfluidic plate and an obround alignment hole to constrain movement of the support bracket along at least one of the perpendicular axes.



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10. The microfluidic cartridge of claim 1, wherein:  
the frame has a substantially rectangular opening,  
the microfluidic plate sits within the substantially rectan-  
gular opening,  
the substantially rectangular opening has opposing side 5  
walls that face towards one another, and  
the plurality of apertures comprises two apertures located  
in one of the opposing side walls.
11. The microfluidic cartridge of claim 10, wherein the  
frame comprises an overlapping portion that overlaps a 10  
portion of the microfluidic plate.

\* \* \* \* \*

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UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 12,251,702 B2  
APPLICATION NO. : 18/827174  
DATED : March 18, 2025  
INVENTOR(S) : David Elliott Kaplan et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In Column 14, Line 33, the text “the second fluidic ports” of Claim 1 should be deleted and replaced with --the analysis device ports--.

Signed and Sealed this  
Twenty-ninth Day of April, 2025



Coke Morgan Stewart  
*Acting Director of the United States Patent and Trademark Office*



# **EXHIBIT 7**





# AVITI™ System

## User Guide

### FOR USE WITH

AVITI System, catalog # 880-00001

AVITI System LT, catalog # 880-00003

AVITI Operating Software v3.3.0 or later

### ELEMENT BIOSCIENCES

**For Research Use Only. Not for Use in diagnostic procedures.**

Document # MA-00008 Rev. R

April 2025



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CHAPTER 1

# System Overview

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# Introduction

The AVITI System is a next-generation sequencing (NGS) system that provides scalable solutions for high-quality sequencing. The dual flow cell design enables parallel or staggered runs with independent run setup options. The AVITI Operating Software (AVITI OS) offers an abundance of additional features to promote adaptive run setup and streamline analysis.

The AVITI System supports flexibility through multiple sequencing kit configurations, including kits with 2 x 75, 2 x 150, and 2 x 300 read lengths and high, medium, and low levels of output. For a low-throughput option, the AVITI System LT provides the same high-quality results for medium- and low-output sequencing kits.

This guide provides an overview of system components, analysis options, maintenance instructions, configuration settings, and safety information for the AVITI System and the AVITI System LT.

## Site Prep and Safety

Before installation of an AVITI System, ensure your site meets the requirements in the *AVITI System Site Prep Guide (MA-00007)*. Before operating or maintaining the instrument, review the safety and regulatory information in [Safety and Compliance on page 57](#).

The instrument does not contain any user-serviceable parts. Exterior shells enclose the instrument to protect the operator from laser light exposure and mechanical parts. Software and interlocks prevent exposure to hazards, and using the AVITI System in an unspecified manner can compromise these protections.

## Warranties and Services

The purchase of an AVITI System includes a standard one-year warranty. Element offers supplemental procedures, preventative maintenance service, and annual service plans. For more information, visit [elementbiosciences.com/instrument-service-coverage](https://elementbiosciences.com/instrument-service-coverage).

## System Compatibility

The AVITI System is compatible with single-strand DNA (ssDNA) libraries prepared with particular library preparation workflows and that use Element sequencing chemistry. For more information on compatibility, see the [Product Compatibility](#) page on the Element website.

To avoid mixing and matching components from different kit configurations and versions, AVITI OS validates the compatibility of the cartridge and flow cell provided in each sequencing kit.

## AVITI System LT Compatibility

The AVITI System LT is compatible with the same workflows and library types as the AVITI System. However, the AVITI System LT is only compatible with Cloudbreak™ and Cloudbreak Freestyle™ sequencing kits that support low- or medium-output levels.

## Additional Documentation

For run preparation and setup instructions for a specific workflow, see the following guides:

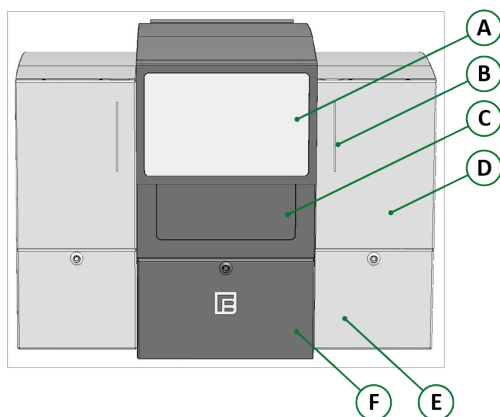
- [Cloudbreak Sequencing User Guide \(MA-00058\)](#)
- [Trinity Sequencing User Guide \(MA-00059\)](#)



## AVITI System Components

The instrument is divided into two sides, side A on the left and side B on the right when facing the instrument. Each side operates independently so you can engage one side while the other is in use. Side A and B each include a dedicated pump bay and reagent bay enclosed with bay doors.

Between sides A and B is the glove-compatible touchscreen monitor that displays the AVITI OS interface. Below the monitor is the nest bay and the waste bay. Lighting illuminates the interior of each bay. During a run, AVITI OS locks all doors except the pump bay doors to protect against laser light exposure, mechanical moving parts, and other hazards.



- A Touchscreen monitor
- B Lightbars
- C Nest bay with automated nest door
- D Pump bays hold fluidic pumps
- E Reagent bays hold reagents for each run
- F Waste bay holds waste bottles

### CAUTION

Do not place items on top of the instrument or on open doors. The doors can support the weight of run and wash components, but applying extra weight or bumping into an open door can damage the instrument.

## Status Lights

The AVITI System includes two types of status lights: an interior nest light in front of each nest and an exterior lightbar on each side. The nest light colors indicate flow cell status. The lightbar colors indicate the current process and overall system status. Unless the system is initializing, each lightbar is side-specific.

## Nest Light Colors

Color	Status
Blue	The flow cell is present and ready to be unloaded.
Green	The flow cell is properly loaded and ready for priming, sequencing, or washing.
Red	The flow cell is improperly loaded: the lid is open or the nest is empty.
None	The flow cell is present but is not ready to be unloaded.



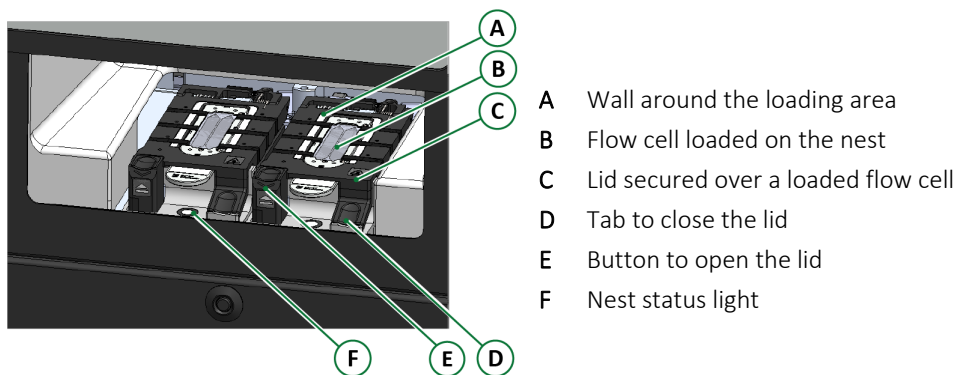
## Lightbar Colors

Color	Status
White fade	The system is initializing.
Solid white	The system is initialized and idle.
Solid blue	Run or wash setup is in progress.
Blue fade	The system is priming, sequencing, or washing.
Solid orange	The system experienced a warning. The color changes after the run finishes.
Solid red	The system experienced an error or run failure. The color immediately changes when an error occurs.

## Nest Bay

The nest bay includes two nests, one for each side, and each nest holds one flow cell. A hinged flow cell lid secures the flow cell in place. A button on each nest unlatches and opens the hinged lid to a 40° angle. To ensure proper alignment, three silver pins on the loading area fit into three corresponding holes on the flow cell cartridge.

An automated nest door on the middle shell encloses the nest bay. During a run, a camera and four tube lenses above the nest image the flow cell in four channels.

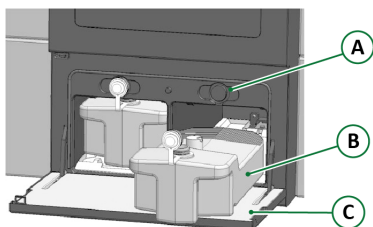


## Waste Bay

The waste bay holds two waste bottles, one for each side. Two threaded cap holders above the waste bay secure the tethered transport cap to keep the caps clear of the door.

A sealed tray built into the bottom of the waste bay collects spills and leaks and directs liquid to the front of the instrument. During run or wash setup, sensors confirm the waste bottle is present and empty and allows the run or wash to proceed. Another sensor detects spills.

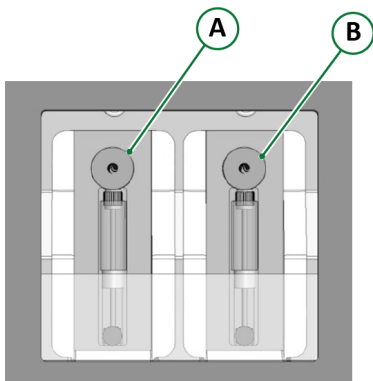




- A Cap holder
- B Waste bottle
- C Open waste bay door

## Pump Bays

Each pump bay contains two pumps that control the flow of liquid. The left pump pulls fluid through the left lane of the flow cell and the right pump pulls fluid through the right lane. Keep the pump bay doors, which allow service access, closed during normal operation and maintenance.

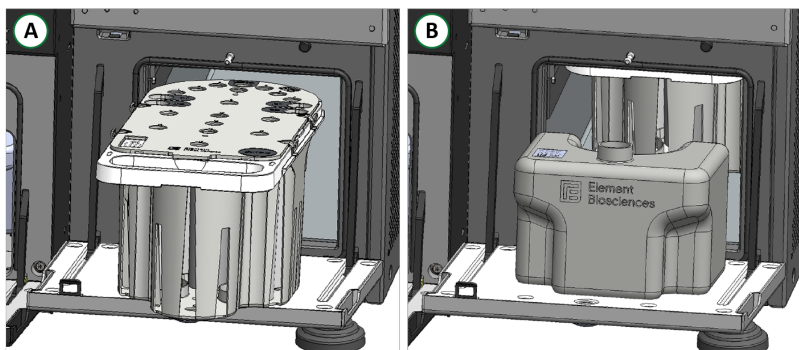


- A Pump controlling the left lane of a flow cell
- B Pump controlling the right lane of a flow cell

## Reagent Bays

Each reagent bay holds a buffer bottle and cartridge basket that contains a cartridge or a wash tray, depending on whether the system is sequencing or washing. Keep the reagent bay doors closed to maintain the refrigeration, which chills reagents.

When priming starts, sippers descend into the bay, pierce the foil seals covering the cartridge wells, and aspirate reagents from the bottom of each well. The sippers continue to aspirate reagents throughout the run. Functioning similarly for a wash, the sippers aspirate wash solution instead of reagents.

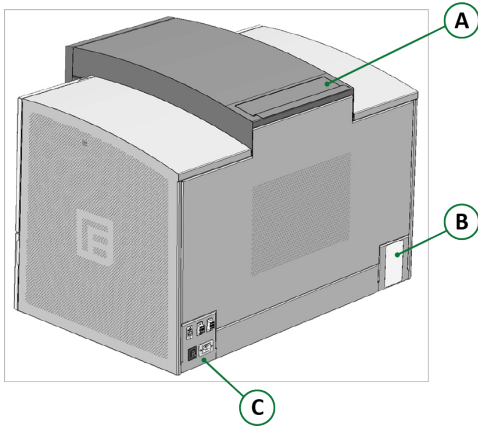


- A Loading a basket and cartridge
- B Loading a buffer bottle



## Back Panel

The back panel includes the air filter tray and input and output (IO) panel. A compliance label displays regulatory symbols for regulatory compliance, the instrument serial number, and electrical specifications. For more information on labeling, compliance, declarations, and certifications, see [Safety and Compliance on page 57](#).



- A Air filter tray
- B Compliance label
- C IO panel

## Air Filter Tray

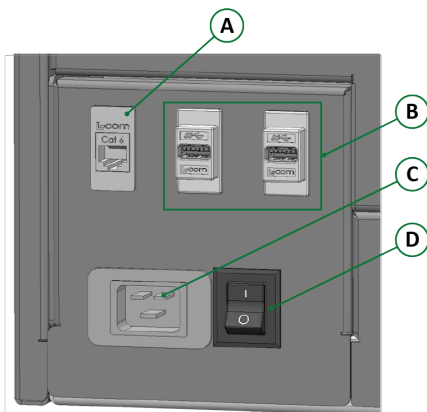
Air enters the instrument through a disposable air filter constructed of pleated paper. The air filter is rated MERV 8, which keeps dust out of the instrument but does not filter smoke or particles < 3 microns. Keeping aerosol and particulate sources away from the instrument extends filter life.

A tray that lifts out of the top of the instrument contains the air filter and facilitates easy replacement. For instructions, see [Replace the Air Filter on page 34](#).

## Input and Output Panel

An IO panel on the back of the instrument groups connections and the power switch. A Category 6 (Cat6) Ethernet port connects an Ethernet cable, and a power entry module connects the power cord. When connecting the instrument to power, use only the power cord that Element provides.

The IO panel also includes two USB 3.0 ports to connect a mouse, keyboard, or drive for transferring files. Side B includes a third USB 3.0 port. A USB drive that transfers files to or from the instrument must be in **FAT32 format**.



- A Cat6 Ethernet port
- B USB 3.0 ports
- C Power entry module
- D Power switch in the on position

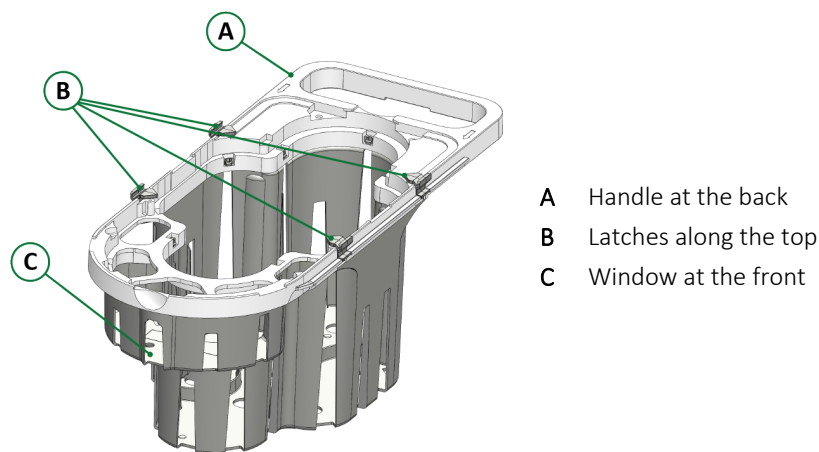


## Reusable Accessories

Cartridge baskets, wash trays, and waste bottles support run setup and washes while minimizing waste. These accessories are reusable but require periodic replacement.

### Cartridge Basket

The cartridge basket protects the cartridge during a run. The back of the basket extends into a handle with arrows that indicate the loading direction. Clips along the top of the basket secure the cartridge. The curved area under the handle accommodates the buffer bottle, which is loaded into the reagent bay behind the basket. A window at the front of the basket enables library inspection.

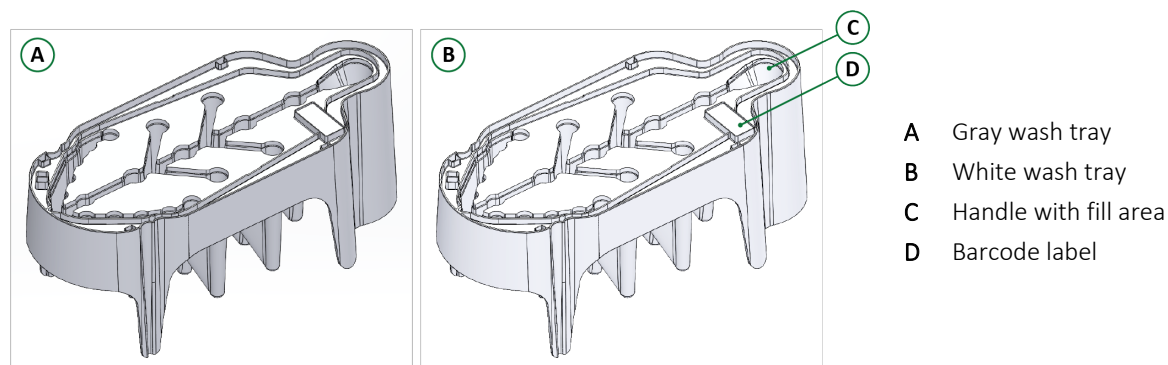


### Wash Trays

The AVITI System includes two types of wash trays, each dedicated to different wash solutions:

- AVITI Wash Tray 1, Gray, for use with Wash 1 Solution.
- AVITI Wash Tray 2, White, for use with Wash 2 Solution and nuclease-free water.

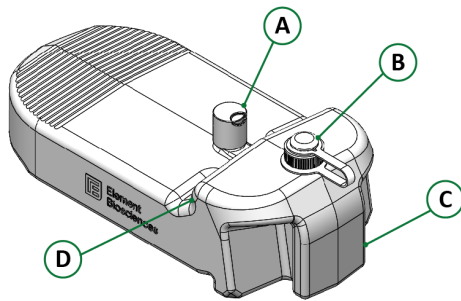
The back of a wash tray forms a handle with a fill area for adding wash solution. Interior fill lines indicate approximate volumes, and an overflow wall contains any wash solution that exceeds the 800 ml maximum fill volume. Each tray includes a water-proof barcode label for validation purposes.





## Waste Bottle

A waste bottle collects spent reagents and library throughout the run. The maximum capacity of 3.2 L per bottle is sufficient to contain all waste from one run. The tethered transport cap seals the bottle during transport. The vent cap improves flow when emptying waste. Ridges on the back of the bottle and a handle at the front facilitate handling.



- A Vent cap
- B Transport cap
- C Handle
- D Thumb indentations



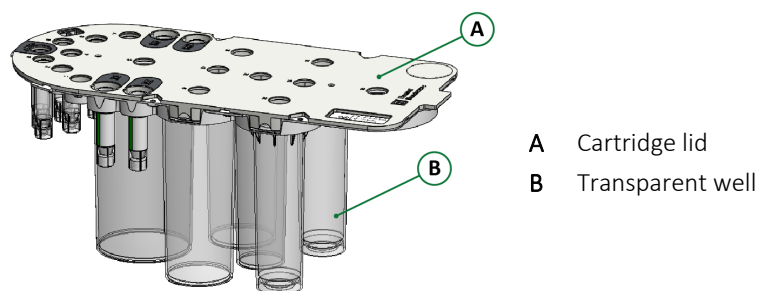
## Sequencing Kits

A sequencing kit provides a flow cell, a reagent cartridge, loading buffer, and wash buffer required for one run. The cartridge for each kit supports a specific number of cycles and output levels. Components includes a barcode label for tracking and validation. To ensure the compatibility of run components, see the [Product Compatibility](#) page on the Element website.

### Sequencing Cartridge

The reagent cartridge is a collection of reagents and buffers in foil-sealed wells that are packaged in an easy-to-load container. The cartridge lid secures the wells and labels the reagent positions. Each well is transparent to allow visual inspection after thawing. A barcode label enables tracking and validation.

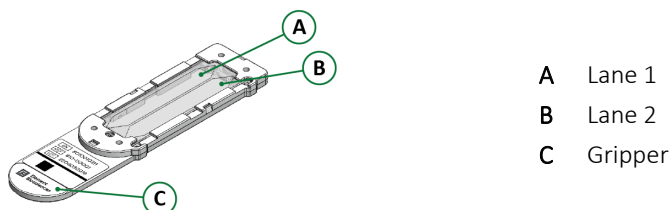
The Library well is reserved for the diluted library. For kits that are compatible with the Individually Addressable Lanes add-on, the AUX well is reserved for a second library. For more information, see [Individually Addressable Lanes on page 17](#).



- A Cartridge lid
- B Transparent well

### Flow Cell

The flow cell is a two-lane glass substrate encased in a plastic cartridge. The cartridge includes a gripper for safe handling. Proprietary surface chemistry coats the flow cell and enables polony generation and sequencing. Library and reagents enter the flow cell through inlet ports and exit as waste through outlet ports.



- A Lane 1
- B Lane 2
- C Gripper

### Loading and Wash Buffers

A sequencing kit includes multiple loading and wash buffers that are packaged separately. Instrument Wash is included in the sequencing cartridge.

Buffer	Packaging	Description
Library Loading Buffer	Tube	The reagent for diluting the libraries to the target loading concentration
AVITI Universal Wash Buffer	Buffer bottle	The reagent that flushes reagents from the flow cell during a run
Instrument Wash	Cartridge	The wash solution for the automatic post-run wash



CHAPTER 2

# Software and Analysis

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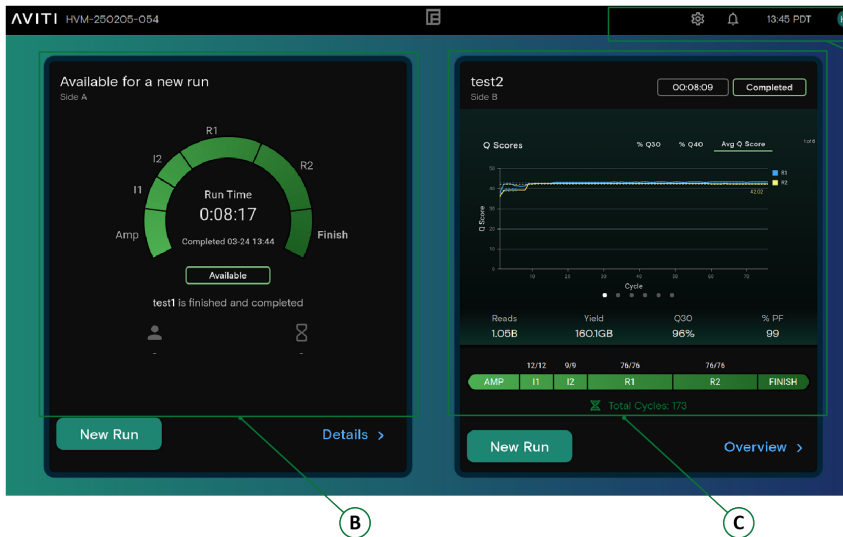
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# AVITI Operating Software

AVITI OS controls instrument operations during sequencing, analysis, and instrument washes. The Home screen functions as a system dashboard, displaying the status of each side with features to start runs and washes and monitor sequencing runs.



- A Taskbar
- B Overview view
- C Details view

## Home Screen Views

The Home screen includes buttons that display the following views:

- **Overview**—Displays general system status and previous run times for each side, or displays active run or wash information.
- **Details**—Displays metrics for an active run. When a run starts, AVITI OS automatically switches to this view.
- **History**—Preserves metrics from the last run. When no run or wash is active, this view is available.

## Taskbar Icons

A taskbar at the top of the Home screen provides the following icons. The Settings and Notifications icons each open a unique screen. USB Drive and User icons each access additional features and functions.

Icon	Name	Function
	USB Drive	View a list of USB drives that are connected to the instrument and safely disconnect a USB device.
	Settings	View system information and configuration settings. See <a href="#">Settings on page 16</a> .
	Notifications	Review notifications and perform the indicated action. See <a href="#">Notifications on page 18</a> .
	User	Open the User menu. Alternatively, this icon displays initials.



## Settings

AVITI OS includes configurable and read-only settings that control the instrument profile and system connections. AVITI OS divides the settings among the following tabs:

- **About**—Displays software and instrument information:
  - » AVITI OS version and the last license acceptance date
  - » AVITI System name, instrument type, serial number, available local storage, and compute ID
  - » Updates available for system firmware and software




### NOTE

Compute ID is a unique code for the integrated circuit that identifies the instrument computer.

- **General**—Controls the system name and displays on-screen keyboard, telemetry, and elevation settings. Also, exports log files from offline systems and resets the air filter time.
- **Network**—Controls network and internet connections for the system. Includes a connectivity indicator.
- **Storage**—Lists storage connections with connectivity indicators and settings for adding and managing storage connections.
- **Add-Ons**—Displays the add-ons enabled on the system and any applicable expiration dates. The tab always appears on offline systems and only appears on online systems with at least one active add-on.
- **User**—Provides password management for offline systems and online systems with local authentication. The tab only appears on applicable systems.



## Network Status

The Network tab displays the following icons, which indicate the status of the network connection. An additional Indicator appears on the tab to show internet connectivity.

Icon	Network Status
	Connected
	Local internet only
	Disconnected

## Storage Status

The Storage tab displays the following icons, which indicate the status of the storage connections. An additional Indicator appears on the tab to show storage connectivity.

Icon	Storage Status
	At least one storage connection
	No storage connection



## Add-Ons

Add-ons enable additional instrument capabilities. The Add-Ons tab displays each add-on available on the instrument. To enable an add-on, contact a sales representative or Element Technical Support.

For online instruments, AVITI OS refreshes the add-on list every 12 hours and when the system restarts. For offline instruments, the installation of add-ons requires additional steps. For more information, see [Install Add-Ons on an Offline System on page 48](#).

## Filter Mask

The Filter Mask add-on modifies the cycles used for filtering, which is advantageous for certain applications. Applying the feature causes run output data and on-instrument run metrics to account for the filter mask.

## High Output Kits

The High Output Kits add-on enables sequencing with high-output kits.

## Individually Addressable Lanes

The Individually Addressable Lanes add-on enables loading one library pool in each lane of a flow cell. The second library is loaded into the AUX well of the sequencing cartridge.

The add-on is only compatible with sequencing kits that meet the following requirements:

- Cloudbreak™ or Cloudbreak Freestyle™ chemistry
- 2 x 75 or 2 x 150 size
- A high-, medium-, or low-output designation

### CAUTION

The Individually Addressable Lanes add-on is **not** compatible with any 2 x 300 size sequencing kits, the Cloudbreak UltraQ™ sequencing kit, or Trinity sequencing kits. The cartridges in these kits reserve the AUX well for other reagents and cannot accommodate a second library.

## PMG Shift

Polony map generation (PMG) refers to the process of mapping polonies during a sequencing run. The PMG Shift add-on enables the skipping of up to 20 cycles for compatibility with particular sequencing runs for Adept™ or third-party libraries. Skipped cycles do not affect data output. For more information on the applicability of this add-on, contact Element Technical Support.

## ElemBio Catalyst

ElemBio Catalyst™ is a native cloud storage and analysis subscription service within ElemBio Cloud. ElemBio Catalyst allows Element to host and manage cloud storage connections on your behalf. Your data is stored in Amazon Simple Storage Service (Amazon S3) storage buckets that are completely dedicated to you.

To use ElemBio Catalyst, purchase an ElemBio Catalyst subscription or subscribe to a 45-day free trial. If your ElemBio Catalyst storage connection is disabled, an **Expired** badge is displayed for 14 days and the run storage connection cannot be used to upload runs. After 14 days, the ElemBio Catalyst storage connection is no longer visible in AVITI OS. To resubscribe to ElemBio Catalyst, contact Element Technical Support at [support@elembio.com](mailto:support@elembio.com). For more information, see the [ElemBio Catalyst documentation](#) in [Online Help](#).





## Polony Density

The Polony Density add-on allows users to opt for an increased read output that is prioritized over the highest quality reads and lower error rates. When this add-on is enabled, it is available in the Advanced Run Settings form while you set up a run.





## Notifications

Notifications display system messages across three tabs: General, Side A, and Side B. Expand a notification to see the message, date, and time.

Notification	Icon	Description	Action
Success		A run or wash completed successfully.	Acknowledge successful completion.
Information		The software is ready to be updated to a new version.	Acknowledge the update.
Warning		The system requires your attention, but you can continue operation.	Acknowledge the warning and resolve it by the indicated date.
Error		The system has malfunctioned and requires action to proceed.	Follow the onscreen prompt.

## Unread Notifications

Notifications include badges that indicate the number of unread messages. Checkboxes mark notifications as read or unread. Marking a notification as read can reset the status lights on that side of the instrument.

Icon	Name	Action
	Mark as read	Mark the selected notifications as read.
	Mark as unread	Mark the selected notifications as unread.

## Filtering and Sorting

Notifications include filters with sorting from newest to oldest or oldest to newest.

Filter	Description
All	View all messages on the selected tab.
Read	View only read messages on the selected tab.
Unread	View only unread messages on the selected tab.



## Run Start Options

AVITI OS includes the following options for starting a run:

- **Single start**—Set up and start a run on one side of the instrument.
- **Dual start**—Concurrently set up and start runs of the same run type on both sides of the instrument.
- **Flexible start**—Set up and start a run or recovery wash on a side of the instrument while a run is active on the other side.

AVITI OS allows sequencing with different kits on each side. Because the sides share a camera, the setup of one run can increase the duration of the other run.

## Flexible Start

Flexible start safely pauses the active run and initiates a run or recovery wash on the other side of the instrument. When setting up the second run, AVITI OS finds a safe pause point before proceeding. While the run is paused, set up and start a run or recovery wash on the other side. The runs on both sides proceed asynchronously. For a flexible start recovery wash, the run on the other side proceeds concurrently.

When you initiate flexible start, AVITI OS indicates the typical wait time for the current run step. Pausing the first run typically takes several minutes but can take as long as ~2 hours, depending on the run step. AVITI OS also includes options to cancel flexible start and resume the active run.

For more information on flexible start wait times, see the run setup instructions in the user guide for your sequencing kit.

## Wash Setup Screens

Initiating a wash opens a series of wash setup screens that guide you through setting up a maintenance, standby, or recovery wash. Wash setup functions similar to run setup, but closing the door validates the wash tray presence.

## Run Setup Screens

When you initiate a run, AVITI OS guides you through a series of run setup screens. Each screen provides a set of steps and indicates run setup progress. AVITI OS unlocks the reagent and waste bay doors at the appropriate steps and prompts the loading of consumables. Closing a door validates the presence of each consumable and scans the consumable barcode. The software presents an alert if consumables are expired. A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.

After the step to empty waste and reload the waste bottle, priming starts automatically. Priming prepares reagents for delivery and pumps air and reagents through a used flow cell and the fluidic tubes, preventing contamination between runs.

Run setup steps differ based on the type of kit you are using. For detailed run setup instructions, see [Additional Documentation on page 6](#).



## Advanced Run Settings

During run setup, selecting the Advanced Run Settings button displays settings for additional features that let experienced users modify primary analysis and run recipe configurations. Available features depend on your kit selection, run type, and available add-ons.

Feature	Description
Custom Recipes	Tailors a run execution in consultation with Element. A recipe governs the stages of a sequencing run, so custom recipes can impact specifications and increase run times. The setting provides two options for recipes: <ul style="list-style-type: none"><li>• <b>Preloaded recipe:</b> Select a recipe on the instrument, such as the short insert or long insert custom recipes. To ensure run compatibility, contact Element Technical Support.</li><li>• <b>Uploaded recipe:</b> Element creates an encrypted, custom recipe package as a .rec file, which you upload from a USB. To obtain a .rec file, contact Element Technical Support.</li></ul>
Filter Mask	Sets the mask for the Filter Mask add-on. See <a href="#">Filter Mask on page 17</a> .
PMG Shift	Sets the number of cycles skipped for the PMG Shift add-on. See <a href="#">PMG Shift on page 17</a> .
Polony Density	Relaxes certain quality filters to increase the total number of polonies in a run. The setting has two options, with Standard as the default option. The High Density option increases the read output. This feature is also known as Expert Mode HD.

## Signing In and Out

Signing in to AVITI OS requires the email address and password for your organization. The first time you sign in to AVITI OS after instrument installation or an update, you must accept the license agreement. A Logout option on the User menu signs you out.

If requested, Element can enable local authentication mode for an online system. This feature assigns a fixed user name and user-defined password to sign in.



## Run Manifest

AVITI OS uses a run manifest as an input file that stores run information, including demultiplexing settings, settings for FASTQ files, and a list of samples with any corresponding index sequences. After a sequencing run, AVITI OS provides the run manifest as an output file to support run analysis and Bases2Fastq.

The run manifest uses a comma-separated values (CSV) file format and can be created using a template on the [Resources page](#) of the Element Biosciences website. For more information on creating a run manifest, see the [Run Manifest Documentation](#) in the [Online Help](#).

## Default Run Manifest

When a sequencing run does not include a run manifest, AVITI OS generates a default run manifest that assigns all reads to one sample during FASTQ file generation.

Demultiplexing indexed libraries is **not possible** with a default run manifest. To use a default run manifest with Bases2Fastq, you must edit the file and create a corrected run manifest that includes sample and index information.

## Run Manifest for Individually Addressable Lanes

If you are using the Individually Addressable Lanes add-on, the Lane column in your run manifest must correctly associate samples with both library pools.

- Lane 1 refers to the library pool loaded into the Library well of the sequencing cartridge.
- Lane 2 refers to the library pool loaded into the AUX well of the sequencing cartridge.

For an example run manifest set up for the Individually Addressable Lanes add-on, see [Sample Specification Examples](#) in the [Online Help](#).



## Analysis Overview

Onboard primary analysis software calls bases, assigns quality scores (Q scores), and generates run metrics. The software extracts and corrects intensities from images to call a base, then assigns a Q score to the base to indicate confidence in the call.

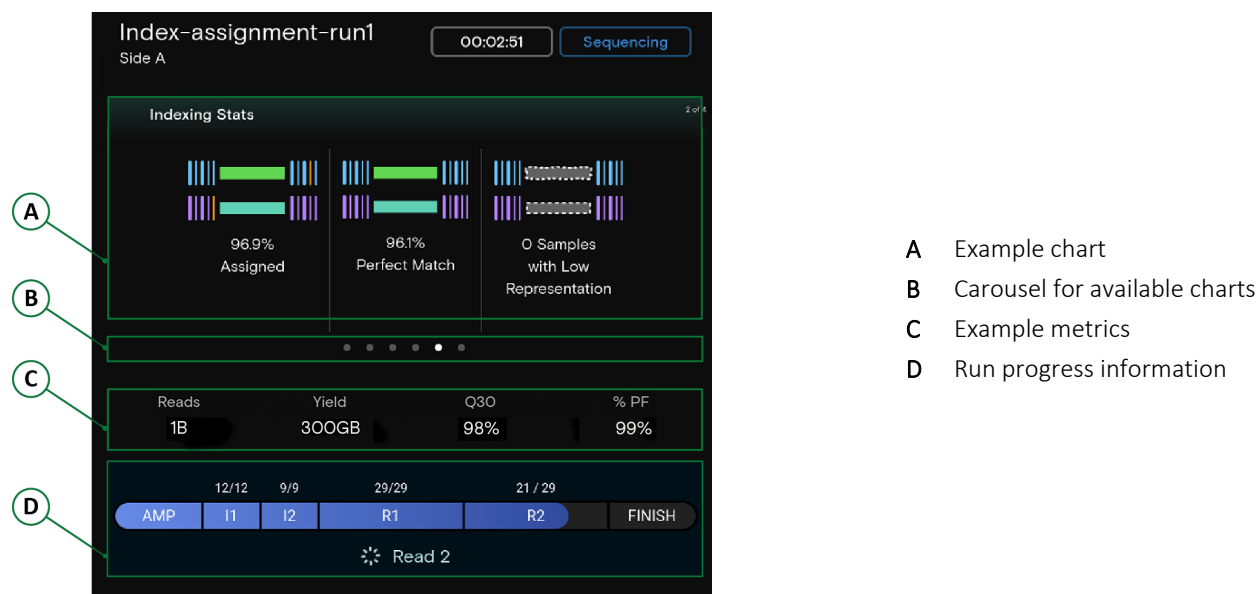
## Run Monitoring

During a run, the Details view displays initial estimates for primary-analysis-generated run metrics that monitor overall run health and progress. As the run progresses, metrics appear and regularly update. Runs that use the Individually Addressable Lanes add-on display metrics and charts for each library pool. The metrics are included in the run output and remain onscreen until you set up a new run.

The metrics and charts for a run depend on your workflow. Additional metrics appear in the charts, which you can cycle through. For more information on the types of metrics and charts that can appear during a run, see [Sequencing Metrics and Charts](#) in the [Online Help](#).

### NOTE

To obtain final metrics for a run, execute the Bases2Fastq Software after the run completes. For more information, see the [Bases2Fastq Documentation](#) in the [Online Help](#).



## Thumbnail Image

The thumbnail image displays a snapshot of the colonies on a tile from the first cycle in the run. If you are using the Individually Addressable Lanes add-on and two library pools, AVITI OS displays an image for each pool.

The image indicates sample density and loading concentration on the flow cell. You can use the image as a quality check to identify overloaded or underloaded flow cells.

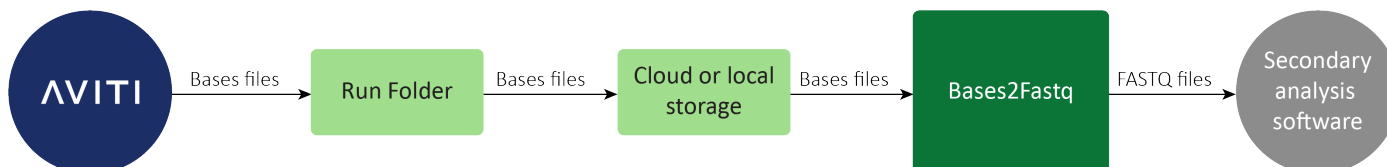


## Run Output and Storage

The output of a run is the run folder, which contains bases files with genomic data and other run data. Bases files are the primary output of a run. A storage connection transfers the run folder from the instrument to your storage location, which can be local or in the cloud. For more information, see [Storage Connections on page 36](#).

The run folder for a sequencing run contains bases files with genomic data and other run data. Bases files are the primary output of a sequencing run. An AvitiRunStats.json file serves as the source file for run metrics. You can set up the integration of metrics into another system, such as a LIMS interface. For more information on output files, see [Sequencing Run Output Files](#) in the [Online Help](#).

After a run, use Bases2Fastq to perform demultiplexing and convert the bases files into FASTQ files for secondary analysis with the third-party software of your choice. For more information, see the [Bases2Fastq Documentation](#) in the [Online Help](#).



### Run Folder

A run folder is named for the run name and contains the run output files, including an AvitiRunStats.json file that serves as the source information for run metrics. You can leverage the data in this file to set up the integration of metrics into another system, such as a LIMS interface.

For more information on the run folder contents, see [Sequencing Run Output Files](#) in the [Online Help](#).

### Local Disk Storage

Because the system software transfers runs to off-instrument storage locations, local disk storage is intended only for temporary storage. Accordingly, the instrument hard drive has sufficient space to store at least two runs and start an additional two runs. When you initiate run setup, AVITI OS checks whether the system has sufficient space to support the run. If AVITI OS indicates that the system does not have sufficient space, contact Element Technical Support.



# Telemetry

Separate from the transfer of genomic data to your storage location, **which Element cannot access**, telemetry sends instrument health data to Element. These data help support maintenance and troubleshooting and do not include any confidential information.

Telemetry is limited to the following data:

- **Software metrics**—Software and firmware versions, CPU and memory metrics, and the instrument serial number, ID, and name. These metrics are communicated as part of regular telemetry events.
- **Hardware metrics**—Data on motors, fans, lasers, and other instrument hardware, which helps Element understand the probable condition of select hardware components.
- **System logs**—Routine logs the system generates when idle or running. The logs include power cycle times, errors, internal communications, and the status of internal services.
- **Primary analysis metrics**—Run metrics, including data for Q30 scores, error rates, cell confluency, cell and target counts, expression levels, and index assignment metrics. Index assignment and other data exclude sample names.
- **Run information**—Data communicated for a run, including run name and ID, run side, run start and end dates and times, run type (sequencing or washing), consumable information, and the number of cycles per read or batch. The data exclude run descriptions.
- **Run logs**—Run-specific information from a subset of system logs. Data include recipe execution, the timing of run steps, and communications between software, firmware, and hardware.



## ElemBio Cloud

ElemBio Cloud is a central online platform that provides real-time remote run monitoring, data analysis, and account management for Element instruments, including AVITI Systems. Any system in online mode automatically connects to the platform. ElemBio Cloud allows you to connect to cloud service providers for data storage and initiate data analysis automation through flows.

You can access ElemBio Cloud on a computer or mobile device to support your organization from anywhere. For more information, see the [ElemBio Cloud Documentation](#) in the [Online Help](#).

## ElemBio Cloud Metadata

By default, AVITI OS sends the following metadata to a secure and customer-specific ElemBio Cloud database:

- Run description
- Sample names from the run manifest, if applicable

Metadata populate the run monitoring pages in ElemBio Cloud, which is separate from the telemetry database. Therefore, telemetry does not collect metadata. If you prefer to keep metadata on the instrument, contact Element Technical Support and request Restrict Metadata mode. When the mode is enabled, a lock appears on the run description in ElemBio Cloud and sample names are masked as numbers. The numbering reflects the order of samples in the run manifest.



CHAPTER 3

# Maintenance

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Perform a Standby Wash .....	32
Replace the Air Filter .....	34



## Maintenance Schedule

AVITI OS on the AVITI instrument requires a maintenance wash every 30 days. The software provides a warning 2 days before a maintenance wash is due.

Procedure	Frequency	Purpose
Maintenance wash*	Monthly	Cleans the outside of the sippers and prevents microbial growth and particulate debris from accumulating in the fluidic system.

Element recommends the following maintenance schedule for optimal performance.

Procedure	Frequency	Purpose
Power cycle	Weekly	Reinitializes the system and resets the instrument computer, which helps maintain instrument performance.
Standby wash*	Preparing for an idle period of $\geq 7$ days	Prepares one or both sides for an idle period of $\geq 7$ days.
Air filter replacement	Every 6–12 months	Ensures proper cooling and continuous operation. The optimum frequency depends on lab cleanliness.
Exterior cleaning	As needed	Wipe the exterior with a damp microfiber cloth and Simple Green. Avoid harsh chemicals and abrasives.

\* To perform a wash after stopping a run, see [Stop an Active Run on page 52](#).

For a list of maintenance consumables, see the *AVITI System Site Prep Guide (MA-00007)*.

## Wash Tray Maintenance

Keep the wash trays in good condition to maximize time between replacements and prevent cross-contamination.

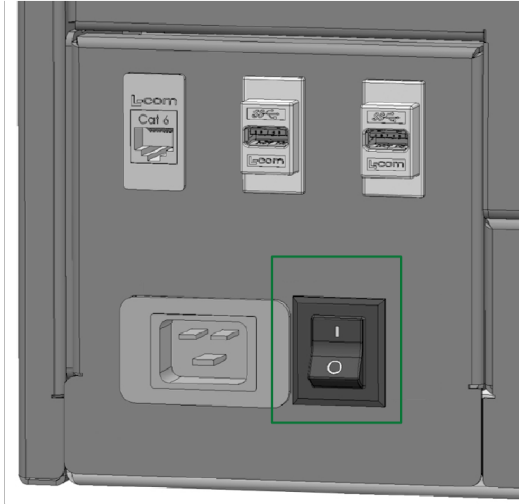
- After each use, discard residual wash solution, rinse the wash tray with nuclease-free water, and air-dry upside down.
- Store clean and dry wash trays upside down. Stack up to two wash trays.



## Power Cycle the System

A power cycle resets the instrument computer, safely shutting down and restarting the system to maintain performance or recover from a problem. Turning off the system without a proper power cycle is reserved for emergencies.

1. Select the user menu, and then select **Shut Down**.
2. When prompted, select **Shut Down** again to shut down the instrument computer.
3. Wait for the screen to go blank and a No Signal message to appear.
4. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.



5. Wait **10 seconds** to make sure the system fully shuts down.
6. On the IO panel, press the power toggle switch to turn on the instrument.  
—The system initializes and displays the Home screen.—
7. If a USB drive is connected to the instrument, reconnect it:
  - a. In the taskbar, select **USB Drive**, and then select **Eject**.
  - b. Detach the USB drive from the instrument.
  - c. Reconnect the USB drive to the instrument.  
—Reconnecting the USB drive allows AVITI OS to detect it after a power cycle.—



## Perform a Maintenance Wash

The maintenance wash is a two-part wash that takes a total of ~1.5 hours. Wash 1 cleans the system, removing residual library and carryover. Wash 2 rinses the system, removing residual Wash 1 solution and preparing for the next run. Each wash requires specific volumes of freshly prepared wash solutions.

### Prepare Wash Solutions

1. Gather the following materials:
  - » 2 L bottles (2)
  - » 4.00–4.99% sodium hypochlorite
  - » Gray wash tray
  - » Nuclease-free water
  - » Pipette controller
  - » Serological pipettes (2)
  - » Tween 20
  - » Used flow cell
  - » White wash tray

—A used flow cell might already be present on the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 37.5 ml 4.00–4.99% sodium hypochlorite to the bottle to prepare 1.54 L ~0.12% sodium hypochlorite.
5. Label the bottle **Wash 1 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 1 Solution at room temperature. Use within the day or discard.
8. Add 1.5 L nuclease-free water to a new 2 L bottle.
9. Attach a new serological pipette to the pipette controller.
10. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
11. Label the bottle **Wash 2 Solution**.
12. Cap the bottle and invert several times to mix.
13. Set aside Wash 2 Solution at room temperature.

### Initiate a Maintenance Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a **used** flow cell:
  - a. Select **Open Nest**.
  - b. Place the used flow cell onto the nest and close the lid.
  - c. Select **Close Nest**.
3. Select which side to wash:
  - » **Side A**—Set up a maintenance wash on side A.
  - » **Both**—Set up maintenance washes on sides A and B.

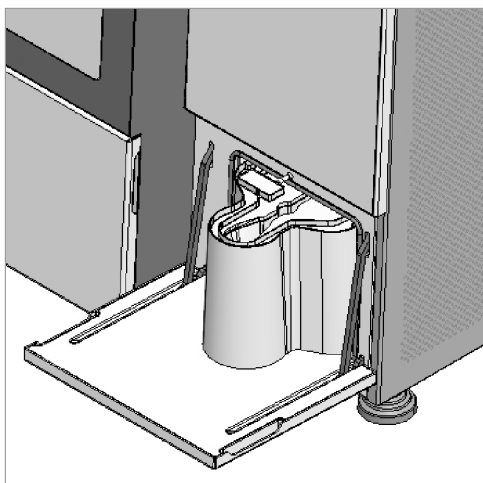


» **Side B**—Set up a maintenance wash on side B.

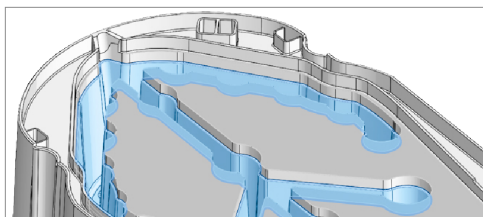
4. Select **Wash**, and then select **Maintenance**.
5. Select **Next** to proceed to the Load Wash 1 screen.

## Load Wash 1 Solution

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered gray wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.



5. Add 590 ml freshly prepared Wash 1 Solution to the fill area, filling the wash tray to slightly above the lower fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. Select **Next** to proceed to the Empty Waste screen.

## Empty Waste and Run Wash 1

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

### CAUTION

Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

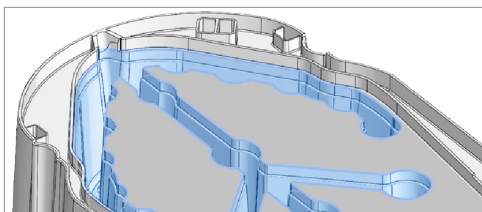
4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
  - a. Position the bottle over the funnel or waste receptacle.



- If you inserted a funnel, align the handle to the inner edge of the funnel.
  - If you did not insert a funnel, center the handle over the waste receptacle.
  - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
  - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
  8. Screw the transport cap onto the cap holder and close the waste bay door.
  9. Select **Next** to open the Run Wash 1 screen and automatically start the wash, which takes ~34 minutes.
  10. During the wash, process the materials removed from the reagent bay:
    - » If you removed a used buffer bottle and cartridge basket, follow the discard instructions in the user guide for the kit.
    - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 27](#).
  11. When the wash is complete, select **Next** to proceed to the Load Wash 2 screen.

## Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the gray wash tray from the reagent bay and set aside.
  - Residual liquid in the wash tray is normal.—
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops and close the reagent bay door.
7. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.

## Run Wash 2

1. Select **Next** to open the Run Wash 2 screen and automatically start the wash, which takes ~52 minutes.
2. When the wash is complete, select **Done** to return to the Home screen.
3. Leave all materials in the instrument.
4. Process the gray wash tray from the first wash per [Wash Tray Maintenance on page 27](#).



## Perform a Standby Wash

A standby wash takes ~52 minutes and flushes nuclease-free water through the fluidic system, removing any residual Tween 20. When complete, the washed side is idle. Performing a maintenance wash on the idle side ends the idle period and enables sequencing.

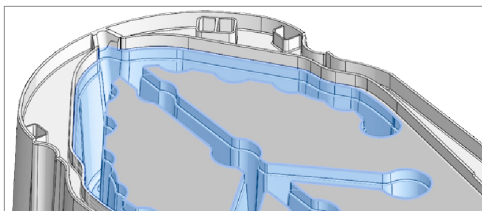
### Initiate a Standby Wash

1. Gather the following materials:
  - » Nuclease-free water
  - » Used flow cell
  - » White wash tray

—A used flow cell might already be present on the instrument.—
2. On the Home screen, select **New Run**.
3. If AVITI OS prompts that the flow cell is missing, load a **used** flow cell:
  - a. Select **Open Nest**.
  - b. Place the used flow cell onto the nest and close the lid.
  - c. Select **Close Nest**.
4. Select which side to wash:
  - » **Side A**—Set up a standby wash on side A.
  - » **Both**—Set up standby washes on sides A and B.
  - » **Side B**—Set up a standby wash on side B.
5. Select **Wash**, and then select **Standby**.
6. Select **Next** to proceed to the Load Water screen.

### Load Nuclease-Free Water

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.



6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door.
8. Select **Next** to proceed to the Empty Waste screen.



## Empty Waste and Run the Standby Wash

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

### CAUTION

Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
  - a. Position the bottle over the funnel or waste receptacle.
    - If you inserted a funnel, align the handle to the inner edge of the funnel.
    - If you did not insert a funnel, center the handle over the waste receptacle.
  - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
  - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Water screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Remove Tray screen.

## Unload the Wash Tray

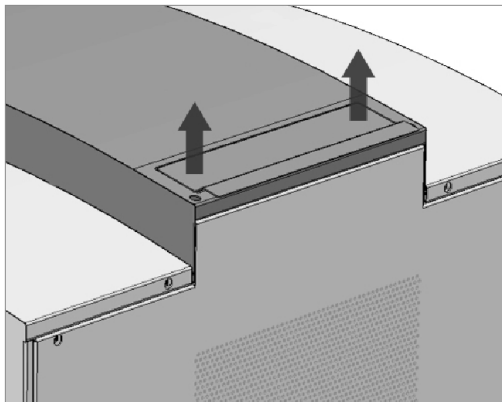
1. When prompted, open the reagent bay door and remove the wash tray.
  - Residual liquid in the wash tray is normal.—
2. Close the reagent bay door.
3. Select **Done** to proceed to the Home screen.
4. Leave the flow cell in the nest.
5. Process the materials removed from the reagent bay:
  - » If you removed a used buffer bottle and cartridge basket, follow the discard instructions in the user guide for the kit.
  - » If you removed a wash tray, follow the guidelines in [Wash Tray Maintenance on page 27](#).



## Replace the Air Filter

To ensure proper cooling and continuous operation of the system, replace your air filter every 12 months. If your site is located at a high elevation, replace your air filter every 6 months. For more information, see the *AVITI System Site Prep Guide (MA-00007)*.

1. If the instrument sequencing or washing, wait for the run or wash to complete.
2. Select the user menu, and then select **Shut Down**.
3. When prompted, select **Shut Down** again to shut down the instrument computer.
4. Wait for the screen to go blank and a No Signal message to appear.
5. On the IO panel on the back of the instrument, press the power toggle switch to turn off the instrument.
6. Using the flange toward the back of the instrument, lift the air filter tray out of the top.



7. Remove the air filter from the tray and discard.  
—The filter might be loose in the tray, which is normal.—
8. Place the tray on a table or benchtop.
9. With the small arrow on the side of the filter pointing up, place the new air filter into the tray.
10. Lower the tray into the instrument. Use the pins to align the tray to the rails and guide entry.
11. On the IO panel, press the power toggle switch to turn on the instrument.  
—The system initializes and displays the Home screen.—



CHAPTER 4

# System Configuration

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## System Connections

The AVITI System uses a combination of network, internet, and storage connections to operate. Each system requires a network connection and at least one storage connection. Cloud storage connections, telemetry, over-the-air software updates, and remote support require an internet connection.

Mode	Network Connection	Internet Connection	Storage Connection
Online	Internet	DHCP or static	Cloud or local
	Local	DHCP or static	Local
Offline	Local	None	Local

## System Modes

The system mode determines connection options and settings for exporting log files, password protection, and software updates:

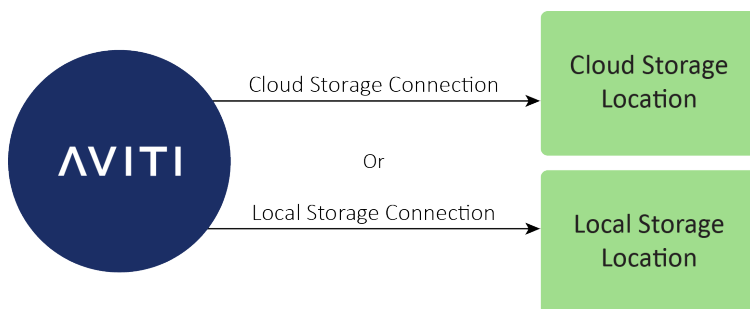
- Online mode connects the system to the internet, which streamlines operations.
- Online local authentication mode operates in online mode but includes local authentication, which avoids network requirements to allowlist Auth0 IP addresses. Only an Element representative can enable this mode.
- Offline mode operates the system without an internet connection. Only an Element representative can enable offline mode.

## Storage Connections

A storage connection establishes an off-instrument location that AVITI OS transfers files to. Each run delivers bases files and other run outputs to the default storage location unless you specify a different location during run setup.

AVITI OS supports cloud and local storage connections:

- A cloud storage connection transfers files to a storage location in the cloud.
- A local storage connection transfers files to a storage location on a local network or USB drive.



## Storage Connection Requirements

Adding a storage connection requires permissions, network information, and account information that your IT administrator can provide. You must set up cloud storage connections in ElemBio Cloud and fulfill the requirements for the cloud service provider.

For comprehensive storage requirements, see the *AVITI System Site Prep Guide (MA-00007)*.



## Supported Storage Connections

Cloud storage connections include ElemBio Catalyst, Amazon Web Services (AWS), DNAnexus, and Google Cloud Storage (GCS). For local storage, AVITI OS supports Server Messenger Block (SMB) and USB.

The storage location for a cloud storage connection is a bucket. A connected bucket is available to all systems. Local storage is exclusive to the system.

### Cloud Storage

Cloud Storage Connection	Description
ElemBio Catalyst	<ul style="list-style-type: none"> <li>Subscription-based service.</li> <li>Connects the system to an Amazon Simple Storage Service (Amazon S3) bucket that Element creates and operates on your behalf. For more information, see <a href="#">ElemBio Catalyst on page 17</a>.</li> <li>Transfers data using AWS Identity and Access Management (IAM).</li> </ul>
AWS	<ul style="list-style-type: none"> <li>Connects the system to an Amazon S3 bucket.</li> <li>Transfers data using secret key authentication through IAM.</li> </ul>
DNAnexus	<ul style="list-style-type: none"> <li>Connects the system to a DNAnexus project.</li> <li>Transfers data using an API key for authentication.</li> </ul>
GCS	<ul style="list-style-type: none"> <li>Connects the system to a Cloud Storage bucket.</li> <li>Transfers data using secret key authentication through a keyed-hash message authentication code (HMAC).</li> </ul>

### Local Storage

Local Storage Connection	Description
SMB	<ul style="list-style-type: none"> <li>Connects the system to the server running SMB via a path to a folder.</li> <li>Uses the SMB protocol based on service user authentication to transfer data.</li> <li>Enables import of a run manifest from an SMB storage location during run setup.</li> <li>Supports automatic export of log files from offline systems.</li> <li>Supports Kerberos or NTLMv2 authentication.</li> </ul>
USB	<ul style="list-style-type: none"> <li>Transfers data and log files to a USB drive connected to the instrument.</li> <li>Supports automatic and manual export of log files from offline systems.</li> <li>Supports USB-A 3.0 or newer versions and FAT32 or exFAT formats.</li> <li>Must store ≥ 1.6 TB of data, which is sufficient for at least two full runs.</li> </ul>



## Configure General Settings

General settings include the instrument name setting, the on-screen keyboard setting, file output settings for instrument priming and wash runs, air filter time resetting, and read-only settings that control the instrument profile. For offline systems, general settings also include features to export log files. For instructions, see [Exporting Log Files on page 45](#).

### Name the Instrument

1. On the taskbar, select **Settings**.
2. Select the **General** tab, and then select **Edit**.
3. Enter a preferred name consisting of 1–20 alphanumeric characters, hyphens (-), and underscores (\_) to identify the instrument.  
—The default name is the serial number, field-programmable gate array (FPGA) ID, or Unnamed Instrument.—
4. Select **Save** to apply the name.

### Configure On-Screen Keyboard

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Select the **Show on-screen keyboard** toggle to enable or disable the on-screen keyboard for text-entry fields.

### Configure File Output Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Select the **Save prime output to storage** toggle to enable or disable the output files for instrument priming.
4. Select the **Save wash run output to storage** toggle to enable or disable the output files for a wash run.  
—When you enable the setting, AVITI OS requires you to configure a storage connection before you start a wash.—

### Review Read-Only Settings

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Review the following read-only settings. To change a setting, contact Element Technical Support.

Setting	Default	Description
High Elevation	Disabled	Calibrates the system to operate at a high elevation
Offline Mode	Disabled	Prevents an internet connection

### Reset the Air Filter Time

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. From the Reset Air Filter Time setting, select **Reset**.
4. When prompted, select **Reset** again to confirm that you have replaced your air filter. The timer will be reset.



## Connect to the Network

Network settings connect the system to your network via a Dynamic Host Configuration Protocol (DHCP) or a static IP address. When the system is connected to an Ethernet port, AVITI OS automatically connects to a DHCP server and autopopulates the network settings. Alternatively, you can assign a static IP address and manually configure the network settings.

### Select a DHCP Server

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down menu, select **Automatic (DHCP)**.  
—AVITI OS assigns a dynamic IP address and all other network settings.—

### Assign a Static IP Address

1. On the taskbar, select **Settings**.
2. Select the **Network** tab.
3. In the drop-down menu, select **Manual**.  
—AVITI OS assigns a unique and permanent IP address.—
4. Select **Edit**, and then configure the following network settings.

Setting	Example	Description
IP Address	11.2.34.178	The destination IP address
Gateway	11.2.34.177	The IP address of the gateway computer that manages network communications
Subnet Mask	11.2.34.176	The subnet mask that separates the IP address into host and network addresses
Name Server IP(s)	ngs-1.yourlab.com	The names of up to four Domain Name System (DNS) servers that provide IP addresses

—Two additional network settings, Host name and Mandatory Access Control (MAC) address, are read-only.—

5. Select **Save** to apply the settings and connect to the network.



## Add Storage Connections

The Storage tab lists storage connections for the system, including available storage for each local storage connection. An Element representative adds the first storage connection at installation. After installation, you can add an unlimited number of additional storage connections.

AVITI OS only lets you add local storage connections. To add a cloud storage connection, access ElemBio Cloud. For more information, see [Storage Connections](#) in the [Online Help](#).

## Add an SMB Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. Select **Add Storage**, and then select **Local File Server (SMB)** as the storage provider.
4. In the Name field, enter a preferred name for the storage connection.
5. Complete the following fields to configure an SMB network storage location for the SMB storage connection.

Field	Instruction
Host	Enter the host network IP address or fully qualified domain name (FQDN). The Kerberos authentication protocol requires an FQDN. <ul style="list-style-type: none"> <li>• Example IP address: 1.222.333.44</li> <li>• Example FQDN: datapc.elembio.com</li> </ul>
Port	Enter a port number for the file transfer service or leave blank to accept the default of port 445.
Workgroup/Domain	Enter the name of the workgroup or domain that the user belongs to. The Kerberos authentication protocol requires you to enter the Kerberos realm name. <ul style="list-style-type: none"> <li>• Example workgroup/domain for Kerberos: elembio.com</li> </ul>
Share	Enter the name of the share that makes the directory accessible to SMB.
Path	Enter the path to an <b>existing</b> folder where you want to output data.
User	Enter the user name for the service user.
Password	Enter the password for the service user.

—All fields except Port and Path are required. Certain server configurations require a work group or domain.—

6. In the Temporary Prefix drop-down menu, select **Disabled** or **Enabled**.

—The Temporary Prefix setting appends two underscores to the name of a file (e.g., \_\_ExampleFileName.zip) while in transfer to the SMB location. The prefix disappears when the file transfer finishes.—
7. In the Session Security drop-down menu, select a setting for the level of encryption:
  - » **High (Recommended)**—AVITI OS requests an encrypted connection with the SMB server. This option is the default setting.
  - » **Medium**—The SMB server determines use of an encrypted connection. The server determines if a connection is encrypted or signed.
  - » **Low**—AVITI OS disables extended SMB security negotiation (SPNEGO) for wider compatibility with SMB servers. The SMB server determines if a connection is encrypted or signed.
8. In the File Checksums drop-down menu, select **Disabled** or **Enabled**.

—The File Checksums setting computes the MD5 checksum for each transferred file and lists them in the RunUploaded.json file. You can use this information to verify the integrity of files.—



9. If prompted, select **Confirm** to set the Session Security selection.
10. Select **Save** to add the storage connection.

## Add a USB Storage Connection

For a USB storage connection, the instrument supports USB-A 3.0 or newer versions and the FAT32 or exFAT formats. The USB drive must have > 1.6 TB of available storage space, and the USB name can only use alphanumeric characters, hyphens, and underscores.

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **Storage** tab.
4. Select **Add Storage**, and then select **USB Drive** as the storage provider.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. In the Name field, enter a preferred name for the storage connection.
7. Select **Save** to add the storage connection.  
—AVITI OS makes sure the USB drive is connected to the instrument and has write permission and sufficient storage.—

## Disconnect the USB for a Storage Connection

1. Select **More** for the USB storage connection, and then select **Eject**.
2. Detach the USB drive from the instrument.
3. To reuse the USB after disconnecting, reconnect the device to a USB port.  
—The USB name must remain the same for AVITI OS to identify the storage connection.—



## Manage Storage Connections

Storage settings manage storage connections and include setting the default storage connection. Unless you reset the default storage connection, the default storage connection is the first cloud location added to the instrument. If a cloud location does not exist, the default storage connection is the first local network location.

You can verify any storage connection, but only local storage connections can be edited and deleted in AVITI OS. If you must edit a cloud storage connection, access ElemBio Cloud. For more information, see [Storage Connections](#) in the [Online Help](#).

For ElemBio Catalyst, storage connections that have been expired less than 14 days appear as expired in the storage connections list and cannot be selected in the run setup. Storage connections that have been expired more than 14 days do not appear in the storage connections list. To renew your ElemBio Catalyst subscription, contact your sales representative.

## Verify a Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select **More**, and then select **Verify Storage**.
4. Wait ~20 seconds for a success message to appear, indicating a valid storage connection.  
—AVITI OS indicates that the connection is connected, unverified, or partially verified with a blocked network.—
5. If AVITI OS cannot verify the storage connection, troubleshoot:
  - a. Make sure the storage connection is correctly set up.
    - For an AWS storage connection, check the IAM permissions. See the applicable [JavaScript Object Notation \(JSON\) policy template](#) in the [Online Help](#).
    - For a GCS storage connection, check the role assigned to the HMAC key.
    - For an SMB storage connection, check the permissions associated with the users.
    - For a USB storage connection, make sure the USB is not ejected, and check that the USB name and type are correct. For USB requirements, see [Local Storage on page 37](#).
  - b. If the storage connection is correctly set up, contact Element Technical Support.

## Set the Default Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the applicable storage connection, select **More**, and then select **Set as Default**.
4. When prompted, select **Set Default**.

## Edit a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the local storage connection you want to edit, select **More**, and then select **Edit**.  
—Editing a busy storage connection can affect where run output is stored.—
4. On the Edit Storage Connection screen, edit any of the following fields.



Field	Instruction
Name	Enter a preferred name for the storage connection.
Workgroup/Domain	Enter the name of the workgroup or domain that the user belongs to. The Kerberos authentication protocol requires you to enter the Kerberos realm name. <ul style="list-style-type: none"><li>• Example workgroup/domain for Kerberos: elembio.com</li></ul>
User	Enter the user name for the service user.
Password	Enter the password for the service user.
Session Security	Select <b>High (Recommended)</b> , <b>Medium</b> , or <b>Low</b> .

—The Host, Share, Port, Path, Temporary Prefix, and File Checksums fields are read-only. If you must edit these fields, create another storage connection.—

5. Select **Save** to apply the edits and update the storage connection.

## Delete a Local Storage Connection

1. On the taskbar, select **Settings**.
2. Select the **Storage** tab.
3. For the local storage connection you want to delete, select **More**, and then select **Delete**.
4. When prompted, select **Delete**.

—AVITI OS does not allow you to delete a busy storage connection.—



## Update the Software

AVITI OS checks for new software versions daily and sends a notification when an update is available. The update runs over-the-air and takes 1-2 hours to complete. Make sure that you initiate the update during instrument downtime to avoid disruptions. If an update exceeds 3 hours and you require support, contact ElemBio Support at [support@elembio.com](mailto:support@elembio.com).

For offline systems, Element notifies you of an update and provides the files that are needed for a manual update. Manual updates are only available for systems in offline mode. For instructions, see [Perform a Manual Update on page 48](#).

## Perform an Over-the-Air Update

1. Make sure that the AVITI System is not performing a run or wash.
2. Power cycle the system. For more information, see [Power Cycle the System on page 28](#).  
—For AVITI OS versions 3.3.0 or later, if you haven't performed a system power cycle in the previous 7 days, the system prompts you to power cycle before you start the update.—
3. On the taskbar, select **Settings**, and then select **Update Software**.
4. When prompted, select **Update Now** to start the update.  
—The system might restart multiple times during the update process.—
5. After the update completes, power cycle the system when prompted.
6. After the system power cycles, select **Notifications** to view a notification that confirms success.
7. If the update is unsuccessful or takes longer than 3 hours, contact Element Technical Support.  
—AVITI OS reverts to the previous version so you can continue operation.—



# Manage an Offline System

For AVITI Systems in offline mode, AVITI OS lets you export log files, password-protect the system, and perform manual software updates. To install add-ons, offline systems require a specific procedure that uses a USB with an add-on key downloaded from ElemBio Cloud. These procedures and features are unique to offline mode and help manage offline systems.

## Exporting Log Files

Offline systems support the export of log files using two methods:

- **Automatic export**—Configure AVITI OS to automatically export log files to a local storage location every hour for telemetry purposes. For help connecting exported log files to telemetry, contact Element Technical Support.
- **Manual export**—Export log files to a USB drive as needed to provide troubleshooting resources to Element Technical Support. AVITI OS lets you perform a limited log file export or a full export of log files.
  - » **Limited Log Export**—Export the log files for a selected run. Use to support initial troubleshooting of a run.
  - » **Full Log Export**—Export all log files for a system. Use to support in-depth system and run troubleshooting.

By default, automatic export is disabled and AVITI OS does not export any log files. When exporting log files to a USB drive, a solid-state drive (SSD) offers significant time savings compared to a flash drive.

## Enable Automatic Export of Log Files

1. If necessary, add a local storage connection to export log files to. For instructions, see [Add Storage Connections on page 40](#).
2. On the taskbar, select **Settings**.
3. Select the **General** tab, and then select **Set Up Automatic Export**.
4. In the Storage Connection drop-down menu, select a local storage connection.
5. Select **Save** to enable automatic export.
6. Transfer the exported log files to an internet-accessible location for telemetry.
7. Delete transferred files from the storage location.
  - Each automatic export adds log files to the storage location without replacing or removing existing files.—

## Disable Automatic Export of Log Files

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Disable** to stop automatically exporting log files.

## Change the Automatic Export Location

1. On the taskbar, select **Settings**.
2. Select the **General** tab.
3. Under Export Log Files, select **Edit**.
4. In the Storage Connection drop-down menu, select a local storage location to export log files to.
5. Select **Save** to reset the location.

## Manually Export Full Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.



2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. In the drop-down menu for manual exports, select **Full Log Export**.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. Enter an Export Range using the Start Date, End Date, and time fields.  
—The Export Range cannot exceed 14 days.—
7. [Optional] Select the **All Day** toggle to remove time fields and export all log files for the dates in the Export Range.
8. Select **Export Logs**.  
—AVITI OS exports the log files to the USB drive.—
9. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
10. Detach the USB drive from the instrument.
11. Upload the log files to the location that Element Technical Support provides.

## Manually Export Limited Log Files

1. Connect a USB drive to a USB port on the side or back of the instrument.
2. On the taskbar, select **Settings**.
3. Select the **General** tab.
4. In the drop-down menu for manual exports, select **Limited Log Export**.
5. In the USB Drive drop-down menu, select the USB drive connected to the instrument.
6. In the Export Run drop-down menu, select the run for which you want log files.
  - » Only runs from the last 14 days are available.
  - » If you select an active run, only certain files might be available.
  - » If you attempt to export log files soon after an active run starts, you might receive an error message. Wait until the run progresses further and attempt the export again.
7. Select **Export Logs**.  
—AVITI OS exports the log files to the USB drive.—
8. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
9. Detach the USB drive from the instrument.
10. Upload the log files to the location that Element Technical Support provides.

## Manage Passwords

User settings manage passwords for offline systems and online systems with local authentication. Offline systems support setting, changing, resetting, and removing passwords. An online system supports password reset and removal only.

### NOTE

Resetting or removing a password requires assistance from Element Technical Support.

## Set a Password

1. On the taskbar, select **Settings**.
2. Select the **User** tab.
3. In the Password field, enter a new password.  
—The field accepts  $\geq 4$  alphanumeric and special characters, excluding spaces.—



4. In the Confirm Password field, reenter the new password.
5. Select **Save**.
6. When prompted, select **Yes, Set Password**.

## Change the Password

1. On the taskbar, select **Settings**.
2. Select **User**.
3. In the Current Password field, enter the current password.
4. In the Password field, enter a new password.  
—The field accepts  $\geq 4$  alphanumeric and special characters, excluding spaces.—
5. In the Confirm Password field, reenter the new password.
6. Select **Save** to apply the new password.

## Reset a Lost Password

1. On the login screen, select **Forgot Password**.
2. Select **Generate** to display a password reset token and the instrument serial number.
3. Contact Element Technical Support and provide the token and serial number.  
—Element Technical Support emails you a single-use password reset file.—
4. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
5. Connect the USB drive to a USB port on the side or back of the instrument.
6. Select **Next**.
7. Select **Load Reset File** to upload the password reset file, which removes the password from the system.
8. In the Password field, enter a new password.  
—The field accepts  $\geq 4$  alphanumeric and special characters, excluding spaces.—
9. In the Confirm Password field, reenter the new password.
10. Select **Reset Password** to apply the new password and return to the login screen.
11. Sign in to the system using the new password.
12. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
13. Detach the USB drive from the instrument.
14. Discard the password reset file.

## Remove the Password

1. On the taskbar, select **Settings**.
2. Select **User**, and then select **Remove Password**.
3. When prompted, select **Yes, Remove Password**.
4. Select **Generate** to display a password reset token and the instrument serial number.
5. Contact Element Technical Support and provide the token and serial number.  
—Element Technical Support emails you a single-use password reset file.—
6. Save the password reset file at the root level of a USB drive. Do not rename the file or save it in a folder.
7. Connect the USB drive to a USB port on the side or back of the instrument.
8. Select **Next**.



9. Select **Load Reset File** to upload the password file, which removes the password from the system.
10. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
11. Detach the USB drive from the instrument.
12. Discard the password reset file.

## Perform a Manual Update

1. Unzip the files that Element provides for the update.
2. Save the files at the root level of an exFAT USB drive with a minimum storage of 12 GB.
3. Connect the USB drive to a USB port on the side or back of the instrument.
4. Make sure the AVITI System is not performing a run or wash.
5. Power cycle the system. For more information, see [Power Cycle the System on page 28](#).  
—For AVITI OS versions 3.3.0 or later, if you haven't performed a system power cycle in the previous 7 days, the system prompts you to power cycle before you start the update.—
6. On the taskbar, select **Settings**.
7. Under Software Update, in the USB Drive drop-down menu, select the USB drive that contains the update files.
8. When prompted, select **Update Now** to perform the update.  
—The system might restart multiple times as it updates.—
9. After the update completes, power cycle the system when prompted.
10. After the system power cycles, select **Notifications** to view a notification that confirms a successful update.
11. If the update is unsuccessful or takes longer than 3 hours, contact Element Technical Support.  
—AVITI OS reverts to the previous version so you can continue using the system.—
12. On the taskbar, select **USB Drive**, and then select **Eject** to disconnect the USB drive.
13. Detach the USB drive from the instrument.

## Install Add-Ons on an Offline System

Add-ons on offline AVITI Systems require an installation procedure using a USB with a key downloaded from ElemBio Cloud. The add-on key file must be located at the root level of the USB. For instructions to download the add-on key, see [Add-Ons](#) in the [Online Help](#).

1. Use a USB port on the side or back of the instrument to connect the USB drive with the add-on key file downloaded from ElemBio Cloud.
2. On the taskbar, select **Settings**.
3. Select the **Add-Ons** tab.
4. In the drop-down menu, select the USB Drive.
5. Select **Upload**.  
—AVITI OS uploads the key file, which installs the add-ons. The Add-Ons tab displays the add-ons and expiration dates.—



CHAPTER 5

# Troubleshooting

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# General Troubleshooting

Error messages communicate hardware or software problems and describe both the problem and resolution. General troubleshooting resolves other problems that can occur during system initialization, run setup, and sequencing. If a problem persists, contact Element Technical Support.

A power cycle resolves many common problems. For instructions, see [Power Cycle the System on page 28](#).

Problem	Resolution
After turning on the instrument, the monitor does not display AVITI OS.	Power cycle the system.
The initialization sequence does not complete, and the loading screen persists.	
The software, instrument, keyboard, or mouse stop operating.	
The reagent or waste bay contains liquid.	See <a href="#">Clean Spills and Leaks on page 55</a> .
Liquid is spilling from the front or bottom of the instrument.	
The nest is wet.	
AVITI OS detects a full waste bottle, but the bottle is empty.	Reload the waste bottle and make sure the waste bay is unobstructed.
The reagent bay contains condensation.	Dry the inside of the reagent bay with a clean, dry microfiber cloth. Clean to the back of the bay, avoiding sensors and cables.
The instrument does not accept a USB device.	Confirm that the USB device meets the following requirements: <ul style="list-style-type: none"><li>• Contains &gt; 1.6 TB available space.</li><li>• Uses USB-A 3.0 or a newer version.</li><li>• Uses the FAT32 or exFAT format.</li><li>• Uses a name with alphanumeric characters, hyphens, and underscores only.</li></ul>
AVITI OS does not proceed with run setup despite sufficient storage space on the instrument.	Set up a storage connection to use with the run. A storage connection is required for a run, and on-instrument storage is reserved as a backup in case of network disruption.
A run continues after you stop it.	Wait for the run to stop. AVITI OS waits for a safe point to stop the run, which can take several minutes to ~2 hours depending on the run stage.
Network connection is lost in the middle of a run.	Wait for the AVITI System to reconnect to the network. Sequencing chemistry is not impacted by network disruptions, and the run progresses as expected. After the system reconnects, data transfer resumes. The system has enough local disk storage for two sequencing runs.



Problem	Resolution
Connection to storage location is lost, and the instrument cannot reconnect.	Confirm with your IT department that all necessary ports and URLs in the <i>AVITI System Site Prep Guide (MA-00007)</i> are allowlisted.
The run folder is missing data.	Make sure the user interface indicates that the system is uploading and wait for the upload to complete. <ul style="list-style-type: none"><li>• A slow connection delays data transfer.</li><li>• Data transfer failure prompts a notification.</li></ul>

## Initialization Problems

Power cycle the system to resolve the following initialization problems:

- After turning on the instrument, the monitor does not display AVITI OS.
- The initialization sequence is incomplete, so the loading screen remains after ~10 minutes.



## Cancel Runs and Washes

AVITI OS displays the following buttons for canceling runs and washes:

- **Discard**—Cancels run or wash setup. The button appears when you can discard setup without compromising consumables.
- **Stop**—Appears on the Home screen and cancels an active run. The button is always enabled so you can free the instrument when run parameters are incorrect, data quality is poor, or a hardware problem occurs.

### Discard Run Setup

1. On any run setup screen before priming, select **Discard**.
2. When prompted to confirm the discard, select an option:
  - » **Unlock Door A** or **Unlock Door B**—Discard the run and save the sequencing cartridge.
  - » **Discard Setup**—Discard the run, delete the run, and return to the Home screen without saving the cartridge.
3. If you unlocked the door, proceed with the remaining steps.
4. Open the reagent bay door and remove the cartridge.
5. Place the cartridge on ice or refrigerate at 2°C to 8°C.
6. Place a clean, uncovered white wash tray onto the open reagent bay door.
7. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
8. Add 660 ml nuclease-free water to the fill area, filling the wash tray to slightly above the upper fill line.
9. Slide the wash tray all the way into the reagent bay until it stops.
10. Close the reagent bay door.
  - AVITI OS deletes the run setup and returns to the Home screen.—
11. Set up a new run and use the cartridge within **4 hours**.

### Discard Wash Setup

1. On any wash setup screen, select **Discard**.
2. When prompted to confirm the discard, select **Discard Setup**.
  - AVITI OS deletes the wash setup and returns to the Home screen.—

### Stop an Active Run

Stopping an active run is a two-part process: stop the run, then perform a ~60-minute recovery wash to remove residual library from the fluidic system.

#### CAUTION

Stopping a run is *final*. You cannot resume a stopped run or reuse any of the consumables.

### Stop the Run

1. On the applicable side of the Home or Run Details screen, select **Stop**.
2. When prompted, select **Yes, Stop Run**.
  - AVITI OS finishes the current step, terminates the run, and returns to the Home screen.—
3. Proceed to [Prepare Wash 2 Solution on page 53](#) and complete the recovery wash.



## Prepare Wash 2 Solution

1. Gather the following materials:
  - » 2 L bottle
  - » Nuclease-free water
  - » Pipette controller
  - » Serological pipette
  - » Tween 20
  - » Used flow cell
  - » White wash tray

—A used flow cell might already be present on the instrument.—
2. Add 1.5 L nuclease-free water to a new 2 L bottle.
3. Attach a new serological pipette to a pipette controller.
4. Add 4.5 ml Tween 20 to the bottle to prepare 1.5 L 0.3% Tween 20.
5. Label the bottle **Wash 2 Solution**.
6. Cap the bottle and invert several times to mix.
7. Set aside Wash 2 Solution at room temperature.

## Initiate a Recovery Wash

1. On the Home screen, select **New Run**.
2. If AVITI OS prompts that the flow cell is missing, load a *used* flow cell:
  - a. Select **Open Nest**.
  - b. Place the used flow cell onto the nest and close the lid.
  - c. Select **Close Nest**.
3. Select which side to wash:
  - » **Side A**—Set up a recovery wash on side A.
  - » **Both**—Set up recovery washes on sides A and B.
  - » **Side B**—Set up a recovery wash on side B.
4. Select **Wash**, and then select **Recovery**.
5. Select **Next** to proceed to the Load Wash 2 screen.

## Load Wash 2 Solution

1. Open the reagent bay door.
2. Remove the buffer bottle and cartridge basket from the reagent bay. Set aside both materials.
3. Place a clean, uncovered white wash tray onto the open door.
4. Slide ~2/3 of the wash tray into the reagent bay, so the barcode edge is about flush with the entrance.
5. Add 660 ml freshly prepared Wash 2 Solution to the fill area, filling the wash tray to slightly above the upper fill line.
6. Slide the wash tray all the way into the reagent bay until it stops.
7. Close the reagent bay door, and select **Next** to proceed to the Empty Waste screen.
8. [Optional] Store leftover Wash 2 Solution at 2°C to 8°C for ≤ 2 weeks.



## Empty Waste and Run Wash 2

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

### CAUTION

Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.

4. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
5. Open the transport cap and the vent cap.
6. Support the waste bottle with both hands and empty the waste:
  - a. Position the bottle over the funnel or waste receptacle.
    - If you inserted a funnel, align the handle to the inner edge of the funnel.
    - If you did not insert a funnel, center the handle over the waste receptacle.
  - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
  - c. If necessary, wipe liquid off the bottle.
7. Close the vent cap and return the empty waste bottle to the waste bay.
8. Screw the transport cap onto the cap holder and close the waste bay door.
9. Select **Next** to open the Run Wash 2 screen and automatically start the wash.
10. When the wash is complete, select **Next** to proceed to the Home screen.
11. Discard the sequencing cartridge and buffer bottle and wash the basket. See the discard instructions in the user guide for your sequencing kit.



## Clean Spills and Leaks

Clean the nest, waste bay, or reagent bay to recover from a leak or spill observed when setting up a run or wash. A leak or spill that occurs in the waste bay during a run causes an error and requires cleaning and contacting Element Technical Support.

If the bottom of the instrument is leaking or liquid is spilling from the front of the instrument: shut down and unplug the instrument if doing so is safe and contact Element Technical Support.

### Clean the Nest

1. Dampen a microfiber cloth with isopropyl alcohol.
2. Wipe the nest with the damp microfiber cloth and allow to dry.
3. If necessary, use a polyurethane foam-tip swab to clean additional areas around the nest.
4. Resume run or wash setup.

### Clean the Reagent Bay

1. Keep the reagent bay door open.
2. Remove any materials from the reagent bay and set aside.
3. Wipe the interior of the reagent bay with a damp microfiber cloth, cleaning to the back of the bay while avoiding sensors and cables.
4. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
5. Resume run or wash setup.

### Clean the Waste Bay

1. Keep the waste bay door open. If the leak occurs during a run, open the door:
  - a. Wait for any runs or washes on the unaffected side to finish.
  - b. On the taskbar, select **Notifications**.
  - c. On the applicable error, select **Unlock Waste Module Door**.
  - d. Open the waste bay door.
2. Unscrew the transport cap from the cap holder on the affected side.
3. Remove the waste bottle from the waste bay and close the transport cap.  
**CAUTION**  
Waste droplets might be on the exterior of the waste bottle.
4. Inspect the waste bottle for cracks, holes, and other defects.
5. [Optional] Insert a funnel into a waste receptacle. Make sure the funnel is secure.
6. Open the transport cap and the vent cap.
7. Support the waste bottle with both hands and empty the waste:
  - a. Position the bottle over the funnel or waste receptacle.
    - If you inserted a funnel, align the handle to the inner edge of the funnel.
    - If you did not insert a funnel, center the handle over the waste receptacle.
  - b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.



- c. If necessary, wipe liquid off the bottle.
- 8. Close the vent cap, leave the transport cap open, and set aside the waste bottle.
- 9. Wipe the interior of the waste bay with a damp microfiber cloth.
- 10. Inspect the exterior of the instrument for any visible fluid. If necessary, wipe with a damp microfiber cloth.
- 11. Return the waste bottle to the waste bay.
  - » If the bottle is defective and you have a spare, load the spare.
  - » If the bottle is defective and you do not have a spare, load the defective bottle. Do not use the affected side until the defective waste bottle is replaced.
- A run or wash on either side requires the presence of both bottles.—
- 12. Screw the transport cap onto the cap holder and close the waste bay door.
- 13. Resume run or wash setup. If necessary, set up a new run with new consumables and clean accessories.



CHAPTER 6

# Safety and Compliance

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## General Safety

Review this chapter before operating or maintaining the AVITI System to ensure safe, correct usage. The procedures described in this guide are tested and optimized, so any deviation can compromise results, cause personal injury, or damage the instrument. All personnel operating the instrument must be trained in correct operation and safety.

The *AVITI System Site Prep Guide (MA-00007)* provides delivery information and installation requirements, including instrument specifications, power specifications, and environmental conditions. A field service engineer (FSE) installs the AVITI System.

### WARNING



Do not attempt to move the instrument, which can result in injury. Only trained Element personnel are qualified to safely move the instrument.

## Safety Labels

The following table lists the safety labels affixed to the instrument. The labels identify potential hazards associated with installation, service, and operation. Follow the procedures in this guide as described to avoid interactions that expose you to these hazards.

### WARNING

This product can expose you to chemicals including formaldehyde, which is known to the State of California to cause cancer, and methanol, which is known to the State of California to cause birth defects or other reproductive harm. For more information go to [www.P65Warnings.ca.gov](http://www.P65Warnings.ca.gov).

Potential Hazard	Label	Description
Class 4 Laser	 A yellow rectangular label with a black border. It contains a laser radiation symbol (a triangle with a starburst) on the left. To the right of the symbol, the text reads: "CAUTION CLASS 4 LASER RADIATION WHEN OPEN AVOID EYE OR SKIN EXPOSURE TO DIRECT OR SCATTERED RADIATION" and "PRUDENCE RAYONNEMENT LASER DE CLASSE 4 EN CAS D'OUVERTURE ÉVITER L'EXPOSITION DES YEUX OU DE LA PEAU AU RAYONNEMENT DIRECT OU DIFFUSÉ".	The instrument is a Class 1 laser product that contains a Class 4 laser. See <a href="#">Laser Safety on page 59</a> .
Heat hazard	 A yellow triangular label with a black border. It contains a black symbol of three wavy lines representing heat.	The nest has a hot surface and exposure can cause burns.



## Laser Safety

The AVITI System is certified as a Class 1 laser product per the US Federal Product Performance Standard for Laser Products requirements described in 21 CFR Subchapter J. The exception to these requirements is the deviations described in FDA Laser Notice #56. The product is classified per IEC/EN 60825-1:2014.

### WARNING

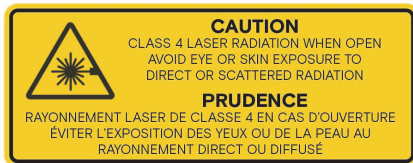
Adjusting or performing procedures other than those described in this guide or other Element guides can result in hazardous radiation exposure.

## Class 4 Laser

The instrument is a Class 1 laser product that contains a Class 4 laser. The Class 4 laser produces Class 4 levels of visible laser radiation, which can be hazardous to eyes and skin. Protective shells and safety interlocks prevent exposure or access to laser radiation levels that exceed Class 1 during operation, maintenance, or normal service.

The following figure depicts the label that identifies noninterlocked portions of the shells that prevent access to laser radiation. Additionally, the nest bay and both reagent bays contain barcode scanners that emit Class 1 levels of laser radiation.

Label identifying noninterlocked locations



## Operating Conditions

Do not operate an AVITI System with bypassed interlocks, damaged shells, or any portion of the shells removed. These conditions make Class 4 levels of laser radiation possible and risk exposure to direct or reflected laser light.

Only Element service personnel, Element-authorized agents, or Element-trained personnel can perform services that require internal interlock bypass or removal of portions of the shells. If you are present during service, take the proper safety precautions to mitigate the risk of direct and reflected laser light.



## Product Compliance

The AVITI System meets the Canadian, EU, South Korean, UK, and US requirements for safety and electromagnetic compatibility (EMC). The system has been tested to and complies with the standards in the following sections.

### US and Canadian Safety and EMC Standards

The AVITI System is certified to the following safety standards:

- IEC 60825-1, safety of laser products
- IEC 61010-1, general safety requirements for electrical equipment for measurement, control, and laboratory use
- IEC 61010-2-010, particular requirements for laboratory equipment for the heating of materials
- IEC 61010-2-081, particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes

The system also has been tested to and complies with the following EMC requirements:

- FCC 47 CFR Part 15, title 47: telecommunication; part 15 – radio frequency (RF) devices
- ICES-003, information technology equipment (including digital apparatus)

### FCC Compliance Statement

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

### EU Safety and EMC Standards

The AVITI System has been tested to and complies with the following safety standards:

- Low Voltage Directive 2014/35/EU
  - » EN 61010-1, general safety requirements for electrical equipment for measurement, control and laboratory use
  - » EN 61010-2-010, particular requirements for laboratory equipment for the heating of materials
  - » EN 61010-2-081, particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes
  - » EN 60825-1, safety of laser products

The system has been tested to and complies with the following EMC standards:

- EMC Directive 2014/30/EU, EMC requirements
  - » EN 61326-1, general EMC requirements for electrical equipment for measurement, control and laboratory use

The system also complies with the Restriction of Hazardous Substances (RoHS) Directive (2011/65/EU) as amended by the Directive (EU) 2015/863. The directives restrict the use of certain hazardous substances in electrical and electronic equipment.

### UK Safety and EMC Standards

The AVITI System has been tested to and complies with the following safety standards:

- S.I. 2016 No. 1101, general safety regulations



- » BS EN 61010-1, general safety requirements for electrical equipment for measurement, control and laboratory use
- » BS EN IEC 61010-2-010, particular requirements for laboratory equipment for the heating of materials
- » BS EN IEC 61010-2-081, particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
- » BS EN 60825-1, safety of laser products

The system also has been tested to and complies with the following EMC standards:

- S.I. 2016 No.1091, EMC requirements
  - » BS EN IEC 61326-1, general EMC requirements for electrical equipment for measurement, control and laboratory use

## South Korea EMC and Regulatory Compliance

The AVITI System has been tested to and complies with the following EMC standards:

- KS C 9610-6-2, electromagnetic immunity standards for equipment used in industrial environments
- KS C 9610-6-4, electromagnetic emission standards in industrial environments

Additional regulatory information to comply with South Korean regulations (in Korean and English):

- 이 기기는 업무용 환경에서 사용할 목적으로 적합성평가를 받은 기기로서 가정용 환경에서 사용하는 경우 전파간섭의 우려가 있습니다.
- "This equipment has been evaluated for its suitability for use in a business environment. When used in a residential environment, there is a concern of radio interference."

## Regulatory Markings

The following markings indicate that the instrument complies with conformity requirements, including EMC and safety requirements, for Australia, Canada, the EU, South Korea, the UK, and the US.

Symbol	Description
	Nemko Electrical Safety Certification Mark for US and Canada
	Australia Regulatory Compliance Mark
	European Conformity (CE) Marking
	UK Conformity Assessed Marking
 R-R-EB6-710-00413	South Korea Conformity Assessment Marking



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# Document History

Revision	Description of Change
April 2025 Document # MA-00008 Rev. R	<ul style="list-style-type: none"><li>• Updated the General tab description in Settings.</li><li>• Updated the maintenance wash requirement.</li><li>• Added ElemBio Catalyst to Supported Storage Connections.</li><li>• Added ElemBio Catalyst to Add-Ons.</li><li>• Added Polony Density to Add-Ons.</li><li>• Updated the estimated completion time for software updates.</li><li>• Added recommendation to perform software updates during downtime.</li><li>• Updated title of Perform a Remote Update to Perform an Over-the-Air update and added power cycle steps.</li><li>• Updated USB drive specifications for manual software updates.</li><li>• Added power cycle steps for manual software updates.</li><li>• Added replacement intervals for air filters.</li><li>• Updated Nemko symbol in Regulatory Markings.</li></ul>
January 2025 Document # MA-00008 Rev. P	<ul style="list-style-type: none"><li>• Removed description of cartridge shipping configurations, such as shipping locks or shipping cover.</li><li>• Removed run setup, sequencing, and thumbnail image troubleshooting. See the <i>Cloudbreak Sequencing User Guide (MA-00058)</i>.</li></ul>
December 2024 Document # MA-00008 Rev. N	<ul style="list-style-type: none"><li>• Added list of additional documentation for workflow instructions.</li><li>• Added description of PMG shift.</li><li>• Added Trinity to the list of kits that are not compatible with individually addressable lanes.</li><li>• Specified that Cloudbreak cartridges include shipping locks and Trinity cartridges include a thermoform cover.</li><li>• Specified that metrics generated during the run are initial estimates.</li><li>• Removed run setup, consumables list, and workflow information to the <i>Cloudbreak Sequencing User Guide (MA-00058)</i>.</li><li>• Removed metrics and replaced with link to the online help.</li><li>• Removed the lid from wash tray description.</li></ul>
August 2024 Document # MA-00008 Rev. M	<ul style="list-style-type: none"><li>• Added power cycle recommendation for instrument software updates.</li><li>• Added base composition information to primary analysis metrics.</li><li>• Updated Individually Addressable Lanes add-on information for clarity.</li><li>• Updated power cycle and air filter replacement instructions.</li><li>• Updated library loading instructions for clarity.</li><li>• Updated system compatibility information.</li><li>• Updated usage statement on front cover.</li><li>• Updated Advanced Run Settings add-on information for clarity.</li><li>• Reorganized loading concentration recommendations to improve clarity.</li><li>• Reorganized flexible start information for clarity.</li><li>• Reorganized materials list into appendix.</li></ul>



Revision	Description of Change
June 2024 Document # MA-00008 Rev. L	<ul style="list-style-type: none"> <li>• Added the AVITI 2x150 Sequencing Kit Cloudbreak UltraQ (catalog # 860-00018).</li> <li>• Added instructions to configure advanced run settings.</li> <li>• Added compatibility information for long insert libraries.</li> <li>• Added contact guidance for enabling add-ons.</li> <li>• Added USB drive requirements.</li> <li>• Added instructions for the manual export of log files for a run.</li> <li>• Updated software descriptions to AVITI OS v2.6.0.</li> <li>• Updated metrics to include Q50.</li> <li>• Updated storage connections to only add local connections.</li> <li>• Updated indexing assignment metric information to clarify requirements.</li> <li>• Updated air filter information.</li> <li>• Updated run output files for completeness and clarity.</li> <li>• Updated warranties and service plan information.</li> <li>• Updated read-only settings.</li> <li>• Updated Online Help cross-references to new style.</li> <li>• Updated document history formatting.</li> </ul>
March 2024 Document # MA-00008 Rev. K	<ul style="list-style-type: none"> <li>• Added Cloudbreak Freestyle sequencing kits.</li> <li>• Added Custom Primer Set Cloudbreak Freestyle.</li> <li>• Added Cloudbreak Freestyle PhiX Control, Adept.</li> <li>• Added Cloudbreak Freestyle loading concentration recommendations.</li> <li>• Added Advanced Run Settings add-on.</li> <li>• Added bead-based normalization information.</li> <li>• Added DNAnexus storage connection information.</li> </ul>
Revision	Description of Change
March 2024 Document # MA-00008 Rev. J	<ul style="list-style-type: none"> <li>• Added recovery wash option for flexible start.</li> <li>• Added Session Security list to SMB storage connection settings.</li> <li>• Updated software descriptions to AVITI OS v2.5.0.</li> <li>• Updated custom primer guidance for new primers and third-party libraries.</li> <li>• Updated PhiX control spike-in information for low-diversity libraries.</li> <li>• Updated instructions for adding primers to include guidance for tube removal.</li> <li>• Updated ElemBio Cloud description for new features.</li> <li>• Updated JSON policy template link for AWS storage connections.</li> <li>• Updated waste bottle information and image.</li> <li>• Reorganized run manifest information.</li> <li>• Removed version 1 sequencing chemistry.</li> <li>• Removed AVITI Custom Oligonucleotide Buffer Set.</li> <li>• Removed PhiX Control Library, Elevate.</li> <li>• Removed wash tray removal from recovery wash procedure.</li> <li>• Replaced product compatibility table with link to webpage.</li> </ul>
November 2023 Document # MA-00008 Rev. J	<ul style="list-style-type: none"> <li>• Updated the AVITI System description.</li> </ul>



Revision	Description of Change
November 2023 Document # MA-00008 Rev. H	<ul style="list-style-type: none"> <li>• Added loading concentration recommendations for Adept Rapid PCR-Plus.</li> <li>• Added PhiX Control Library spike-in recommendation for long-read sequencing of high-plex, low-diversity amplicon libraries.</li> <li>• Added run manifest guidance for individually addressable lanes add-on.</li> <li>• Added run manifest compatibility information for AUX well to sequencing instructions.</li> <li>• Added steps to unzip files for manual updates of offline instruments.</li> <li>• Updated software descriptions to AVITI OS v2.4.0.</li> <li>• Updated system compatibility information for Adept Rapid PCR-Plus and low-diversity amplicon libraries.</li> <li>• Updated flexible start overview for clarity on wait times.</li> <li>• Updated document history entries for consistency of style.</li> <li>• Replaced flexible start diagram with table of pause times.</li> </ul>
September 2023 Document # MA-00008 Rev. G	<ul style="list-style-type: none"> <li>• Added AVITI 2x300 Sequencing Kit Cloudbreak and high, medium, and low output level Cloudbreak kits to guide.</li> <li>• Added AVITI LT System information to Introduction.</li> <li>• Added reminder to open nest lid fully when loading flow cell.</li> <li>• Added temporary prefix setting for SMB storage locations.</li> <li>• Added export range step to manual export of log files.</li> <li>• Added instructions for add-on installation on offline system.</li> <li>• Added image thumbnail troubleshooting information.</li> <li>• Added Australia regulatory marking.</li> <li>• Updated software descriptions to AVITI OS v2.3.0.</li> </ul>

Revision	Description of Change
September 2023 Document # MA-00008 Rev. G	<ul style="list-style-type: none"> <li>• Updated AVITI OS settings for instrument type and Add-Ons.</li> <li>• Updated system compatibility for new kits.</li> <li>• Updated loading concentrations for 2x300 sequencing kit.</li> <li>• Updated max cycles, run parameters, and cartridge thaw instructions for new kits and clarity.</li> <li>• Updated custom primers to specify library that requires custom primers.</li> <li>• Updated links to material safety data sheets.</li> <li>• Updated cartridge images for AUX well and added reagents.</li> <li>• Updated primary analysis information for individually addressable lanes.</li> <li>• Moved manual update instructions to offline system information.</li> <li>• Reorganized sequencing kits content and storage information.</li> <li>• Renamed this guide to <i>Element AVITI System User Guide</i>.</li> </ul>



Revision	Description of Change
July 2023 Document # MA-00008 Rev. F	<ul style="list-style-type: none"> <li>• Updated software descriptions to AVITI OS v2.2.0.</li> <li>• Updated run metrics for Q40, Avg Q Score, Thumbnail Image, Base Composition, and reads passing filter.</li> <li>• Updated run setup steps for user interface changes.</li> <li>• Updated bases file name and quantity for run output.</li> <li>• Updated cycle start info for metrics to defer to AVITI OS.</li> <li>• Update software update instructions to indicate potential for multiple restarts during process.</li> <li>• Added indexing assignment and image thumbnail information.</li> <li>• Added references to online help documentation.</li> <li>• Reorganized sequencing sections for user interface changes.</li> <li>• Reorganized product compliance and regulatory markings to include EU, UK, and South Korean compliance.</li> </ul>
June 2023 Document # MA-00008 Rev. E	<ul style="list-style-type: none"> <li>• Added loading concentrations for Cloudbreak chemistry.</li> <li>• Added information on ElemBio Cloud.</li> <li>• Corrected the cartridge map to show position 1 as nonhazardous.</li> <li>• Updated the sequencing basket image.</li> <li>• Updated flow cell loading image.</li> <li>• Updated kit size parameter description for clarity</li> <li>• Updated Kerberos authentication examples for SMB storage connection fields.</li> </ul>
April 2023 Document # MA-00008 Rev. D	<ul style="list-style-type: none"> <li>• Updated software descriptions to AVITI OS v2.0.0.</li> <li>• Updated instructions on custom primers, run setup, reagent disposal, discarding runs, storage connections, and exporting log files.</li> <li>• Updated run statistic population times and added index assignment.</li> <li>• Updated lightbar colors to include washes, warnings, and errors.</li> <li>• Updated descriptions of the Home screen, run stages, settings, telemetry, run manifest, storage connections, and storage locations.</li> <li>• Updated the links for accessing user guides, templates, and safety data sheets.</li> <li>• Added a chemical exposure warning for Proposition 65.</li> <li>• Added an expected wait time for flexible start.</li> <li>• Added custom primer requirements.</li> <li>• Added instructions and a notification for software updates.</li> <li>• Added network and storage status indicators.</li> </ul>



Revision	Description of Change
April 2023 Document # MA-00008 Rev. D	<ul style="list-style-type: none"> <li>• Added a USB storage connection and taskbar icon.</li> <li>• Added the High Elevation setting and removed the Dark Mode setting.</li> <li>• Added Element oligonucleotide contents.</li> <li>• Added troubleshooting for barcode scanning, flow cell compatibility, and index assignment.</li> <li>• Added LoopSeq for AVITI as a compatible library and kit compatibility.</li> <li>• Added the following Element products: AVITI 2x75 Sequencing Kit Cloudbreak (catalog # 860-00004), AVITI 2x150 Sequencing Kit Cloudbreak (catalog # 860-00003), Adept Custom Primer Set (catalog # 820-00009), and Cloudbreak PhiX Control Library, Elevate (catalog # 830-00017).</li> <li>• Identified the reagents in each well of a Cloudbreak cartridge.</li> <li>• Consolidated instructions on replacing primers, denaturing and diluting libraries, and cleaning the waste bay.</li> <li>• Recommended a weekly power cycle.</li> <li>• Moved power cycle instructions from troubleshooting to maintenance.</li> <li>• Renamed AOS to AVITI OS and run statistics to run metrics.</li> <li>• Renamed AOS to AVITI OS, run statistics to run metrics, and the Workgroup field to Workgroup/Domain.</li> <li>• Replaced run.prodstats with AvitiRunStats.json.</li> </ul>
October 2022 Document # MA-00008 Rev. C	<ul style="list-style-type: none"> <li>• Updated software descriptions to AOS v1.2.0.</li> <li>• Updated run statistic population times.</li> <li>• Updated the read counts for approximate run output.</li> <li>• Updated the internet connection for local online networks to DHCP or static.</li> <li>• Updated navigation for the Home, Notifications, and Settings workspaces.</li> <li>• Updated loading concentrations for Adept libraries.</li> <li>• Updated spike-in recommendations for PhiX Control Library.</li> <li>• Updated the buffer bottle design.</li> <li>• Updated instrument certifications and laser labeling.</li> <li>• Updated trademark and patent information in the legal notice.</li> <li>• Renamed the Error Rate tab to PhiX Error.</li> <li>• Renamed the Element Adept Library Compatibility Kit to Element Adept Library Compatibility Kit v1.1.</li> <li>• Renamed the AVITI Sequencing Kit to AVITI 2x150 Sequencing Kit.</li> <li>• Added Element catalog # 860-00002 for the AVITI 2x75 Sequencing Kit.</li> <li>• Added Element catalog # 820-00008 for the Adept Custom Oligonucleotide Buffer Set.</li> <li>• Added custom primer information and instructions.</li> <li>• Added thaw times for the 2 x 75 sequencing cartridge.</li> <li>• Added error handling for exceeding the maximum number of cycles.</li> <li>• Divided the instructions to mix reagents and add library into two procedures.</li> <li>• Specified that primary analysis must be complete before setting up a run.</li> <li>• Replaced Windex glass cleaner with Simple Green All-Purpose Cleaner.</li> <li>• Marked the FAT32 USB drive as optional.</li> <li>• Corrected the list of library dilution consumables.</li> </ul>



Revision	Description of Change
July 2022 Document # MA-00008 Rev. B	<ul style="list-style-type: none"><li>• Updated software descriptions to AOS v1.1.0.</li><li>• Updated reagent thawing, power cycling, and logoff instructions.</li><li>• Updated run folder content: added statistics and upload files, removed log files, and updated bases file extensions.</li><li>• Added requirement to protect the cartridge from light throughout storage, preparation, and run setup.</li><li>• Added a GCS storage connection and an offline mode.</li><li>• Added lightbar colors that indicate system status.</li><li>• Added an onscreen keyboard and removed the requirement to connect a keyboard and mouse.</li><li>• Removed the option to view notifications when signed out.</li><li>• Renamed the settings chapter to <i>System Configuration</i> and reorganized.</li><li>• Clarified the work group definition and requirement for an SMB storage connection.</li><li>• Decreased the priming duration and increased maintenance, standby, and recovery wash durations.</li><li>• Consolidated information on run and wash setup screens.</li></ul>
June 2022 Document # MA-00008 Rev. A	<ul style="list-style-type: none"><li>• Initial release</li></ul>



# Technical Support

Visit the [Documentation page](#) on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

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# EXHIBIT 8



## Specification Sheet

## Element AVITI™ System

Unrivalled combination of cost, quality, and performance that fits any sequencing application at any scale

## Highlights

- Flexibility at a lower cost
- Exceptional accuracy and data quality
- Scalability with a complete range of sequencing kits

## Introduction

Next-generation sequencing (NGS) has revolutionized the field of genomics, empowering researchers to confront complex scientific questions with an evolving portfolio of technology and tools. Despite these innovations, the cost of benchtop sequencing has remained high, requiring factory-scale throughput to achieve any savings. A compromise on cost is often at the expense of quality and flexibility.

The Element AVITI System reimagines the core components of NGS to offer a benchtop platform that grants access to the genomics ecosystem (Figure 1). Delivering flexible throughput at exceptionally low cost, AVITI saves time and resources without the need to batch or accept lesser quality. Avidity base chemistry (ABC) forms the core of a disruptive design that readily adapts to any application, offering methods that scale from amplicon to whole genome, and from short-read to long.

## Scalable experimental design

Whether an experiment requires 2 billion reads per run or 100 million, AVITI enables cost-effective, high-quality sequencing at any scale. Multiple sequencing kit configurations with read lengths of 2 x 75 to 2 x 300 and a full range from high-, medium-, and low-outputs calibrate genomic output without sacrificing cost-effectiveness, even at small scales (Table 1). The cost benefits are similar to a production-scale system without waiting to fill a flow cell or acquire a sufficiently large project.

An alternative to AVITI, the Element AVITI System LT runs both low- and medium-output sequencing kits to offer low-throughput and budget-friendly access to ABC. If future growth and expanded applications require a broader range of throughputs, labs can easily update the AVITI LT to a full-throughput AVITI.

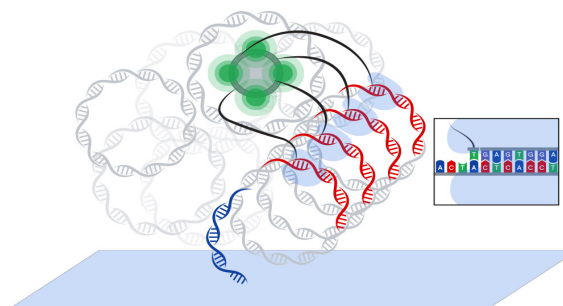


**Figure 1.** AVITI dramatically reduces sequencing costs and turnaround times while elevating the benchmark for genomic data, all in a compact benchtop format that fits into a variety of spaces.

## Novel ABC sequencing

The fundamentals of ABC technology leverage the unique properties of avidites to execute an efficient sequencing reaction and yield highly accurate data. A strong signal-to-noise ratio that persists through high polony densities drives this accuracy.

When a run starts, the library hybridizes to surface primers coating the flow cell. Amplification polymerase then binds to the library and primer duplexes, catalyzing rolling circle amplification (RCA) and generating long DNA strands that include copies of the original library (Figure 2). Each strand forms a polony that contains hundreds of copies of the original library. The polonies hybridize to read-specific sequencing primers.



**Figure 2.** Polymerase binds avidites at the interrogation site. The avidite arms connect to a core that provides a fluorescent signal for detection.





Kit Configuration	Read Length	Output (Gb)	Read Count <sup>a</sup>	Run Time (hours) <sup>b</sup>	Q30
High output	2 x 300	180	300 million	60	> 85%
	2 x 150	300	1 billion	38	> 90%
	2 x 75	150	1 billion	24	> 90%
Medium output	2 x 300	60	100 million	51	> 85%
	2 x 150	150	500 million	31	> 90%
	2 x 75	75	500 million	20	> 90%
Low output	2 x 150	75	250 million	27	> 90%

<sup>a</sup> Performance metrics, including read counts, are based on sequencing Element libraries. Actual results might differ based on factors, such as library type and preparation.

<sup>b</sup> Individually addressable lanes and other custom recipes can extend run times.

**Table 1.** AVITI performance metrics

For each cycle, a sequencing polymerase binds an avidite to a polony and primer duplex, and traps a base-specific avidite to the polony. The result forms an extremely tight complex that enables a 100-fold reduction in reagent concentration compared to sequencing-by-synthesis (SBS).<sup>1</sup>

AVITI and ABC reset expectations on quality scores (Q scores), delivering exceptional Q30 accuracy for 2 x 150 sequencing at > 90% and > 85% for 2 x 300 sequencing. AVITI demonstrated higher accuracy compared to legacy sequencing technology. Data showed fewer soft-clipped reads in difficult homopolymer and repeat regions, among other clear advantages.<sup>2,3</sup>

## Amplification advantages

RCA uses only the original library as a template to avoid magnifying amplification errors. This method avoids incorporating free index primers into colonies and minimizes index hopping on the flow cell. Additionally, RCA delivers more usable reads due to a low rate of optical duplicates, less than 1 %.

## Ordering information

Element AVITI System	880-00001
Element AVITI System LT	880-00003

## References

1. Arslan, Sinan, Francisco J. Garcia, Minghao Guo, et al., "Sequencing by avidity enables high accuracy with low reagent consumption," Nature Biotechnology (May 2023): <https://doi.org/10.1038/s41587-023-01750-7>.
2. Semyon Kruglyak, "Measuring the Accuracy of Element AVITI Sequencing Data," Element Biosciences (blog), July 13, 2022, <https://www.elementbiosciences.com/blog/measuring-accuracy-element-aviti-sequencing-data>.
3. Carroll, Andrew, Alexy Kolesnikov, Daniel E. Cook, et al., "Accurate human genome analysis with Element Avidity sequencing," bioRxiv (August 2023): <https://doi.org/10.1101/2023.08.11.553043>.

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## Summary

AVITI reinvents surface chemistry and data analysis to offer a flexible and cost-effective sequencing platform with exceptional accuracy. Overarching compatibility with standard NGS libraries provides a path to in-house sequencing and accommodates a variety of NGS applications. Multiple kits and abundant software features promote adaptive run setup and analysis to satisfy a spectrum of experiments. AVITI supports your genomic needs.

## System specifications

<b>Instrument Configuration</b>	Dual flow cells AVITI Operating Software Ubuntu Core 20.04 LTS operating system
<b>Operating Environment</b>	Temperature: 18°C to 26°C Elevation: < 2000 m Sound level: ≤ 62 db at 3.3 ft
<b>Instrument Dimensions</b>	(H x W x D) 29.5 in x 37.6 in x 28.5 in Weight: 155.1 kg/342 lb
<b>Power Requirements</b>	100–240 VAC at 50/60 Hz, 15 A. 550 W (average)

To learn more, visit [elementbiosciences.com/products/aviti](https://elementbiosciences.com/products/aviti)



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# EXHIBIT 9



# AVITI

## Discover the AVITI family

Flexible, High-Quality Sequencing and  
Cytoprofiling Solutions

[Watch Demo](#) >

[Our Technology](#) >



## One system, endless possibilities

Our suite of innovative sequencing platforms – AVITI LT™, AVITI™, and AVITI24™ – empowers researchers with cutting-edge sequencing technology, flexibility, and unparalleled performance. Systems are based on our innovative avidite base chemistry and are all compatible with our library preparation solutions, including on-board library conversion, and target enrichment, and Q50 data quality, allowing each system the flexibility to upgrade as your applications and throughput needs grow.

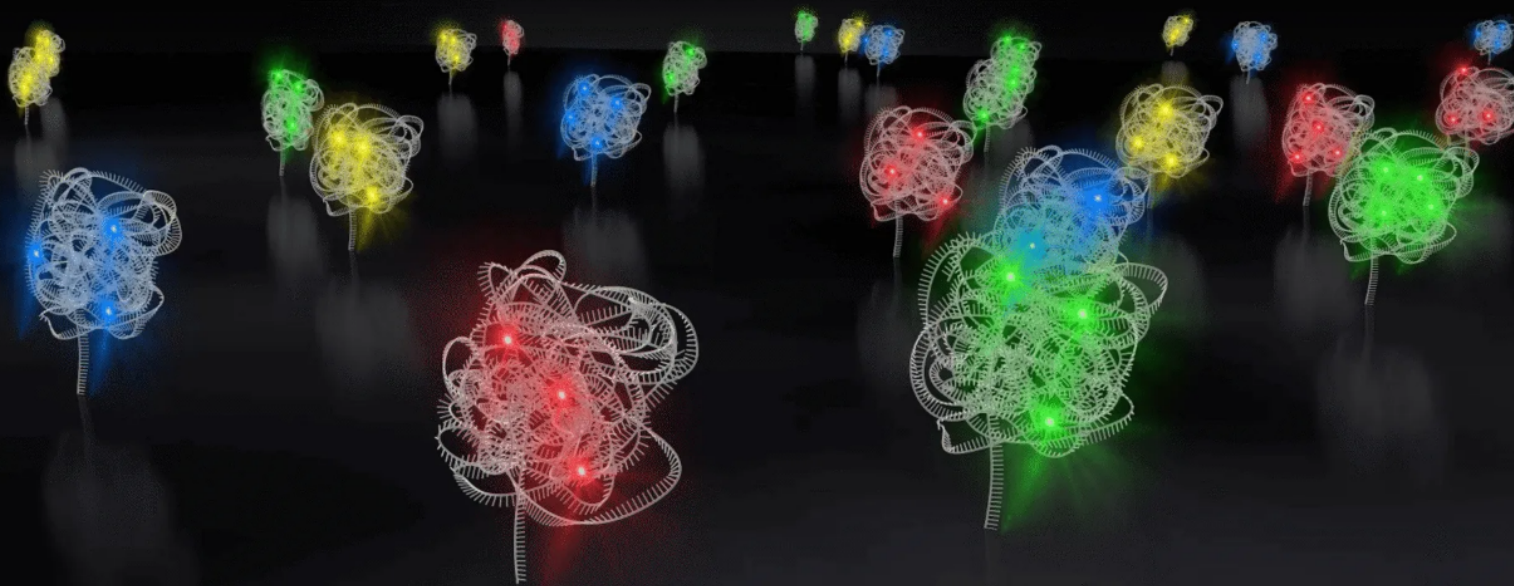
## Powered by Avidite Base Chemistry

Our AVITI line of systems are built on avidite base chemistry (ABC), enabling unparalleled accuracy and efficiency in nucleotide detection. By utilizing a unique combination of enzymatic and chemical processes, ABC enhances the specificity and sensitivity of base calling, resulting in high-fidelity sequencing data with minimal errors. This innovative technology enables researchers to confidently explore the complexities of



utilizing a unique combination of enzymatic and chemical processes, ABC enhances the specificity and sensitivity of base calling, resulting in high-fidelity sequencing data with minimal errors. This innovative technology enables researchers to confidently explore the complexities of the genome, from identifying rare variants to unraveling intricate genetic pathways.

Our Technology >



## AVITI24

AVITI24 brings a new era in scientific exploration by seamlessly integrating state-of-the-art sequencing and CytoProfiling with Teton™ into a single platform. With AVITI24 Teton, you can now analyze DNA, RNA, proteins, phosphorylated proteins, and cell morphology simultaneously from just one sample at single cell resolution.

Learn More >





AVITI

AVITI



## AVITI

The AVITI system conducts cost-effective, high-quality runs across a wide range of applications, without the hassle of batching. Whether it's 100 million reads or 2 billion, AVITI delivers flexible throughput at exceptionally low cost, saving you time and resources without compromising performance. The AVITI can be upgraded to the multiomic capabilities of the AVITI24.

[Learn More >](#)

## AVITI LT

AVITI LT is a low-throughput version of AVITI that brings high-quality ABC sequencing to more users with its lower instrument cost. AVITI LT is the ideal choice for smaller-scale projects or research labs with a small range of applications or limited capital equipment budget. AVITI LT delivers high-quality sequencing with the flexibility to upgrade to the full-throughput AVITI or the multiomic capabilities of the AVITI24.

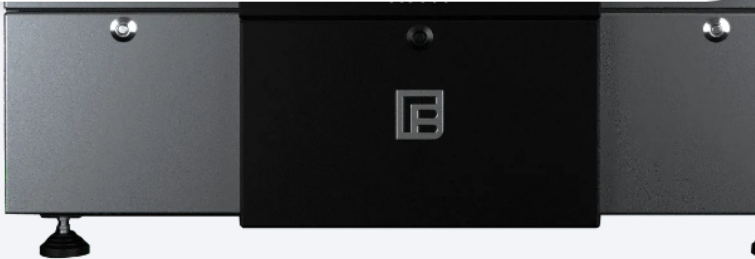
AVITI LT

AVITI



the flexibility to upgrade to the full-throughput AVITI or the multiomic capabilities of the AVITI24.

Learn More >



# Platform Overview

FEATURES	AVITI LT	AVITI	AVITI24
Core NGS Output 500M/FC	✓	✓	✓
Dual Independent Flow Cells	✓	✓	✓
Individually Addressable Lanes	✓	✓	✓
Cloudbreak Freestyle	✓	✓	✓
Cloudbreak UltraQ		✓	✓
Trinity		✓	✓
NGS Quality >90% >Q30 UltraQ: >70% >Q50	✓	✓	✓
High Output NGS 1B/FC		✓	✓
Expert Mode HD		✓	✓
Advanced NGS Run Settings		✓	✓
Teton CytoProfiling			✓
Upgrade Options	Upgrade Available to AVITI or AVITI24	Upgrade Available to AVITI24	N/A





Upgrade Options

Upgrade Available to  
AVITI or AVITI24

Upgrade Available to  
AVITI24

N/A

## Get In Touch

First Name \*

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